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A photographic key to the adult female biting midges (Diptera: Ceratopogonidae: *Culicoides*) of Florida, USA

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Abstract

The biting midges (Diptera: Ceratopogonidae: *Culicoides*) are a diverse group of blood-feeding flies that includes numerous pest and vector species. Major gaps exist in our knowledge of the biology and ecology of the majority of *Culicoides* spp., due in part to a lack of keys for identifying the biting midges of a given region. In Florida, USA, *The Sand Flies of Florida* (Blanton and Wirth, 1979) has been a foundational resource for biting midge identification since its publication. The identification keys to the 47 biting midge species (and one subspecies) in *The Sand Flies of Florida* are not illustrated, however, and frequently rely upon microscopic features (spermathecae, antennal sensory pattern, number of teeth on mandible) as discriminating characters. Here we provide an updated photographic key to 49 nominal species of *Culicoides* from Florida, USA. The revised key orders characters so that species of nuisance, medical or veterinary importance can be reliably identified without slide mounting, an aspect that should facilitate ecological field work. Synoptic tables summarize the taxonomic affinity, distribution, abundance, seasonality, and medical / veterinary importance of the *Culicoides* spp. from Florida, compiled from published sources.

Key words: biting midge, no-see-um, Orbivirus, dichotomous key

Introduction

Culicoides (Diptera: Ceratopogonidae), commonly called biting midges, no-see-ums or sandflies in the USA, are a morphologically and biologically diverse group of blood-feeding flies that includes numerous pest and vector species. The genus *Culicoides* includes approximately 1,350 valid extant species, of which approximately two thirds (875 species) are assigned to subgenus (Borkent and Dominiak, 2021). Despite their importance as serious pests and vectors of diverse pathogens (protozoa, viruses, bacteria, nematodes) of humans, domestic animals and wildlife, major gaps exist in our scientific knowledge of the biology and ecology of most *Culicoides* spp. (Pfannenstiel *et al*. 2015).

Accurate identification of species is a critical step in field research and is hindered by the general lack of keys for identifying the biting midges of a given region (Borkent and Dominiak, 2021). For Florida, USA, Blanton and Wirth (1979) provided detailed keys to the adult females, males, and some pupae and larvae of the 47 biting midge species (and one subspecies) recorded from Florida at that time. The key to adult females in Blanton and Wirth (1979) frequently uses number and shape of the spermathecae, antennal sensory pattern, and number of mandibular teeth as discriminating characters. While these are indisputably reliable diagnostic characters, unambiguous assessment of these features involves specimen clearing, slide mounting, and examination using high magnification (>100x) light microscopy. This process facilitates taxonomic research, but can impede studies of vector incrimination, since large quantities (typically thousands) of adult females must be identified and sorted by species, while maintaining nucleic acid integrity, in order to pool-screen for target pathogens. Identification keys that can avoid slide mounting specimens facilitate ecological and epidemiological research.

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Since the publication of Blanton and Wirth (1979), a few changes to the *Culicoides* fauna of Florida have been reported. *Culicoides jamaicensis* Edwards, has been added to the *Culicoides* fauna of Florida (Wilkening *et al*., 1985). Florida populations of *Culicoides niger* Root and Hoffman (as listed in Blanton and Wirth, 1979) are now considered *Culicoides pallidicornis* Kieffer. *Culicoides variipennis* subspecies *sonorensis* was elevated to species status, *C. sonorensis* (Holbrook *et al*., 2000). Incorporating these updates in a comprehensive key is warranted.

Our objective here is to provide an updated, species-level photographic key for the identification of the adult female *Culicoides* of Florida to facilitate adult biting midge identification by researchers, vector control personnel, and public health workers. The key uses color photographs of external features visible at stereoscopic magnification, when possible, to avoid the need for slide-mounting specimens for viewing with higher magnification (compound / light microscopy).

Materials and Methods

When possible, fresh specimens of many species were collected using carbon-dioxide baited light traps with modifications to capture adults directly into preservative liquid (ethanol or isopropanol) as outlined in Sloyer *et al*. (2019). Wings, bodies, and/or heads of these species were placed in a drop of water on a glass depression slide and photographed using a Nikon DS-Fi3, mounted on a Nikon SMZ-18 Stereomicroscope, and captured using Nikon-Elements D software (Nikon, USA). For specimens on permanent glass slide mounts, images were captured using a digital SLR camera (Canon 6D, Canon USA) with a C-mount adapter and light microscope (Zeiss Primo Star, Zeiss, USA), using a 4x, 10x or 40x objective lens. Multiple (8–20) shallow depth of field (aperture 5.0) images of each target were taken in order to produce a focus-stacked image. The in-focus areas of the images were merged into a single composite image using image-stacking software (ZereneStacker, Zerene Systems, USA). The images were cleaned and cropped in Adobe Photoshop version 21.0.2 (Adobe, USA).

The current key comprises 49 nominal species (Table 1) of *Culicoides* which follows the proposed systematic arrangement of Borkent and Dominiak (2021). Wilkening *et al*. (1985) include 48 biting midge species in their checklist of *Culicoides* in Florida, whereas Grogan *et al*. (2010) report 49 species, without providing a checklist. Two species that have been previously recorded as occurring in Florida are not represented in the current key. *Culicoides eadsi* Wirth and Blanton and *Culicoides luglani* Jones and Wirth were first reported as occurring in Florida in Wirth *et al*. (1985) atlas of wing photographs of Nearctic *Culicoides*. The report of *C. luglani* in Florida (Wirth *et al*., 1985) was determined to be in error (Phillips, 2022), and later corrected (Wirth *et al*. 1988). *Culicoides eadsi*, a species described from Mexico and southern Texas (Wirth and Blanton, 1971)**,** was not included in an annotated checklist of Ceratopogonidae of Florida (Wilkening *et al*., 1985). In their catalog of the New World biting midges north of Mexico, Borkent and Grogan (2009) list *C. eadsi* as occurring in Texas, Florida, Mexico, however no verifiable locality records are known for *C. eadsi* in Florida and the species probably does not occur in Florida.

Couplets and characters used in the current key represent a substantial reorganization of those of Blanton and Wirth (1979). Notably, reliance upon the number of spermathecae in seminal couplets is avoided. Where feasible, subgenera (*Amossovia*, *Avaritia*, *Hoffmania*, *Monoculicoides*) which share key wing characters are clustered together in the key, however utility is given priority over taxonomic grouping in ordering of characters in couplets.

Morphometric measurements follow those of Blanton and Wirth (1979) and Phillips (2022). The proboscis / head (P/H) ratio (Plate 1) is the length of the proboscis from the torma to the tip of the labrum-epipharynx divided by the distance from the torma to the alveolus of interocular seta (bristle). The antennal ratio (AR) of female *Culicoides* is the combined length of the five distal flagellomeres divided by the combined length of the proximal eight flagellomeres. Wing veins (Plate 2) are designated by capital letters with subscript numerals (M_1, M_2, CuA_1, CuA_2) whereas wing cells are designated by lower-case letters with subscript numerals (e.g., r_3, m_1, m_2, cua_1). Major anatomical features and overall morphology of *Culicoides* females are provided in Plate 3.

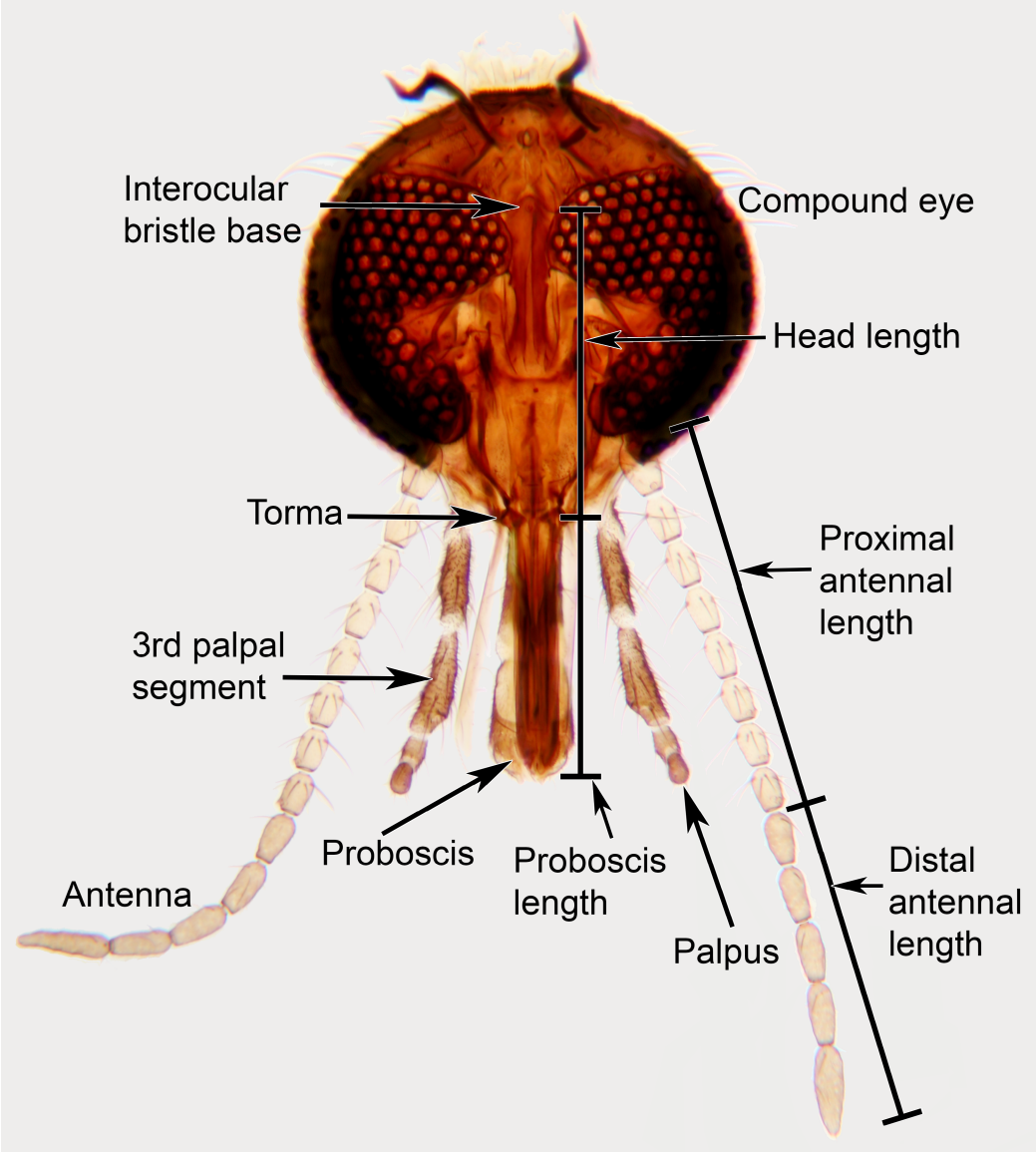

PLATE 1. Head of *C. variipennis* female with major elements labeled.

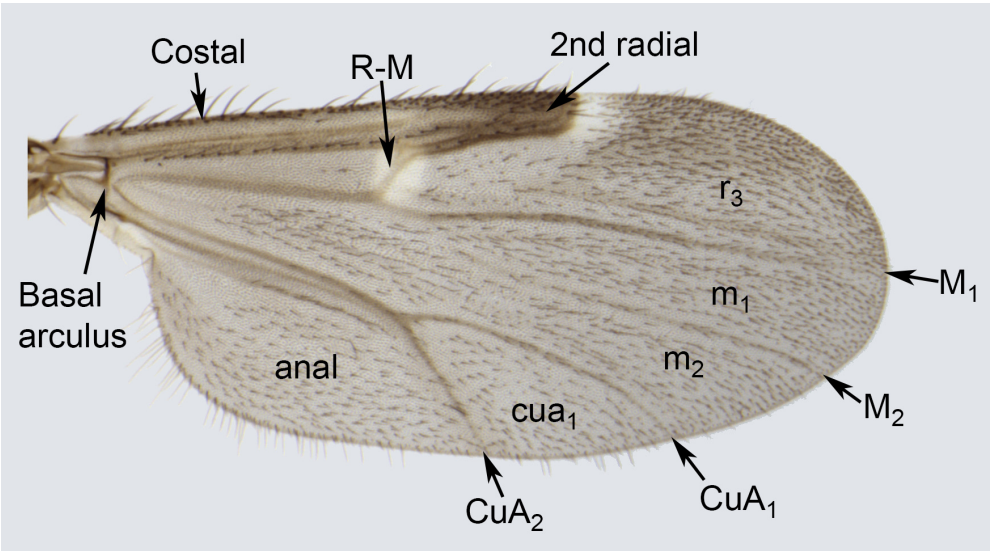

PLATE 2. Wing of *C. biguttatus* female with cells (lower case) and veins (upper case) labeled. Typical pale spots are visible over the R-M crossvein and distal to the 2nd radial cell.

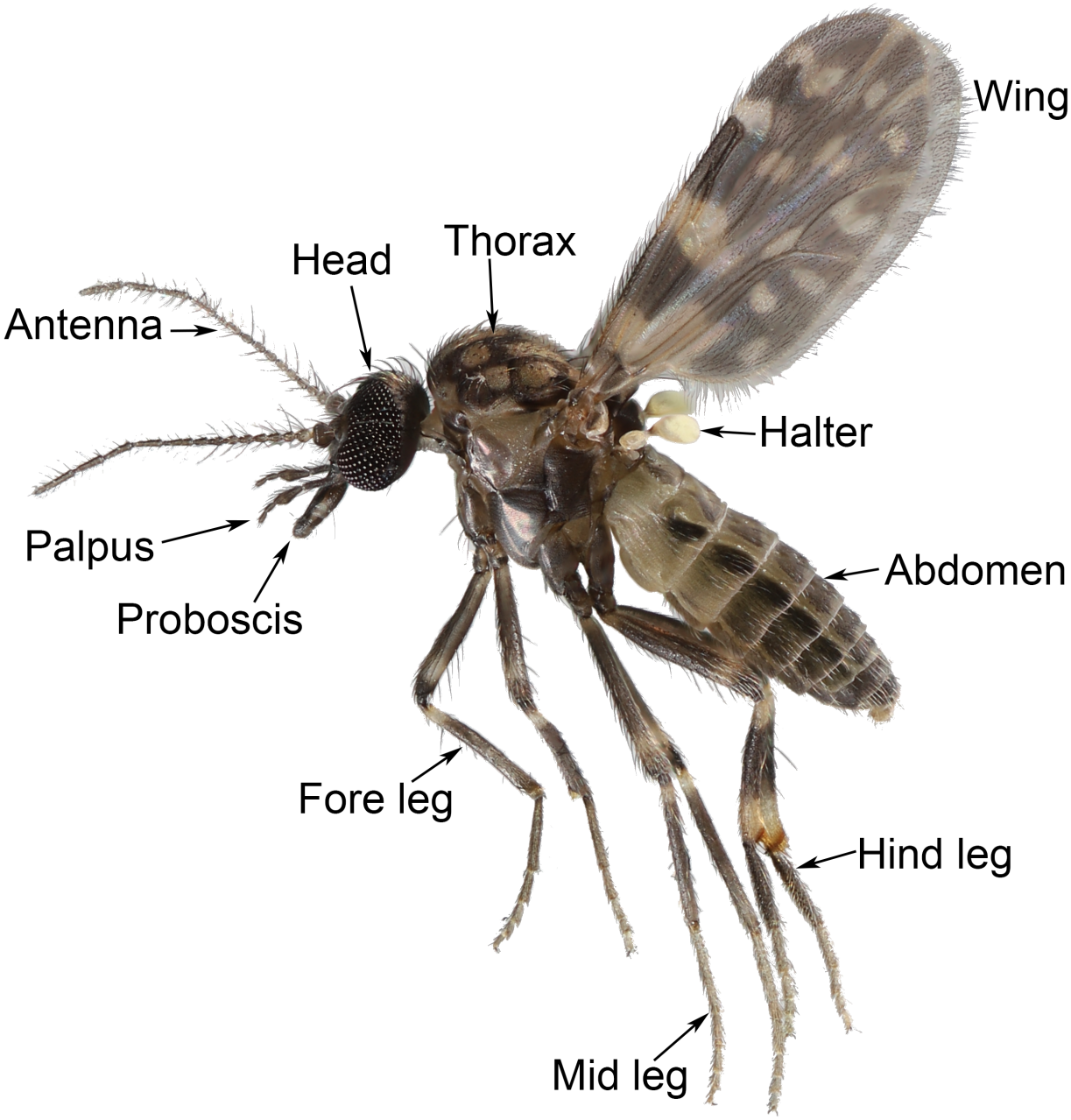

PLATE 3. Habitus of *C. arboricola* female with major features labeled.

Results

Key to species of adult female *Culicoides* of Florida, USA.

1. Cell r_3 with three pale spots beyond 2nd radial cell (Fig. 1, a–d), apical spot occasionally indistinct (Fig. 1–d) 2
   Cell r_3 with two or fewer pale spots beyond 2nd radial cell (Fig. 1, e–h), apical spot sometimes appearing double (Fig. 1-e) . . 7

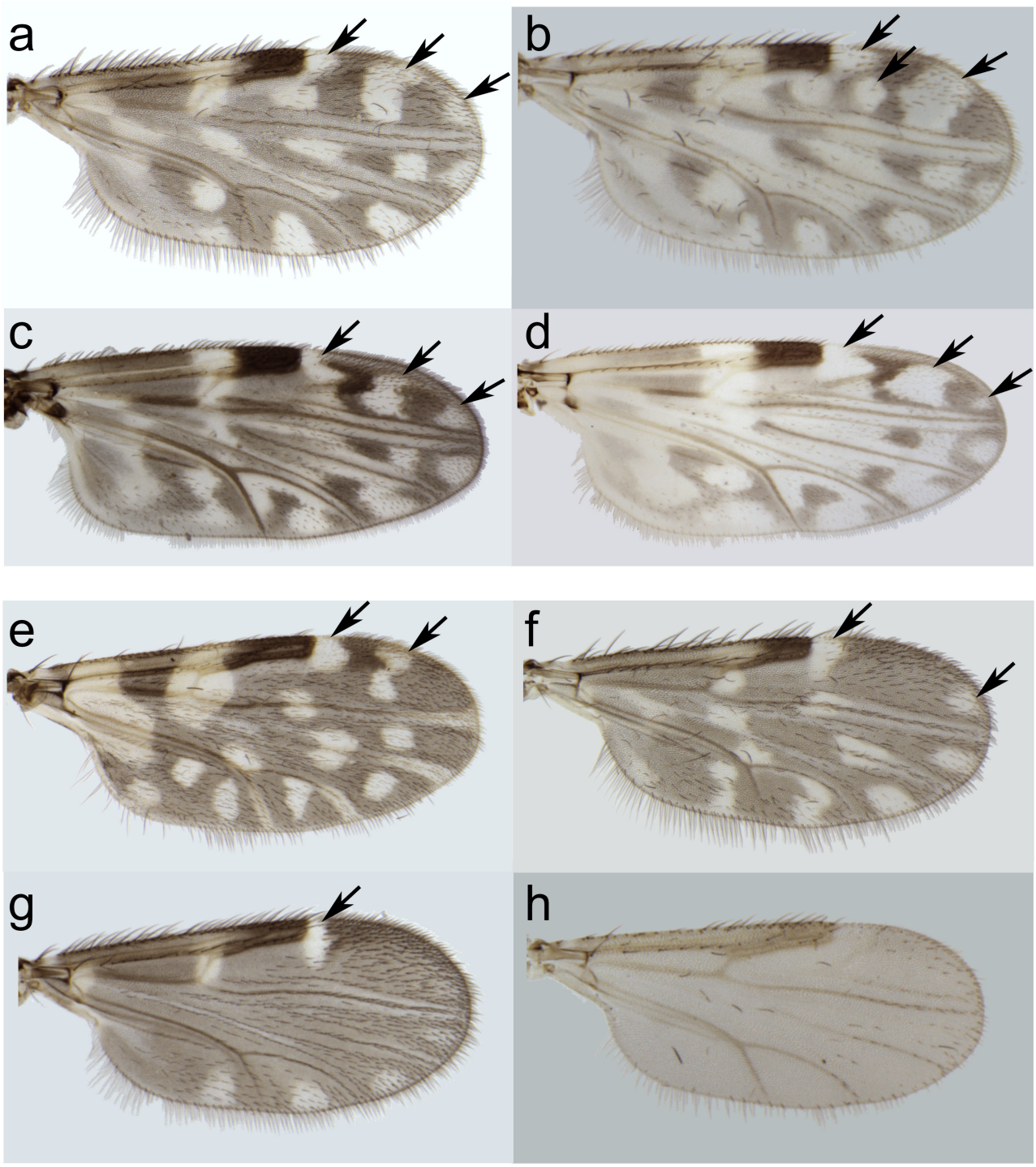

FIGURE 1. (a–h). Wings of *C. stellifer* (a), *C. furens* (b), *C. variipennis* (c), *C. sonorensis* (d), *C. arboricola* (e), *C. haematopotus* (f), *C. nanus* (g), *C. floridensis* (h)

2 (1). Cell cua_1 with pale basal chevron at juncture of veins CuA_1 and CuA_2 (Fig. 2, a–b). 3

Cell cua_1 dark below juncture of veins CuA_1 and CuA_2 (Fig. 2, c–d) 5

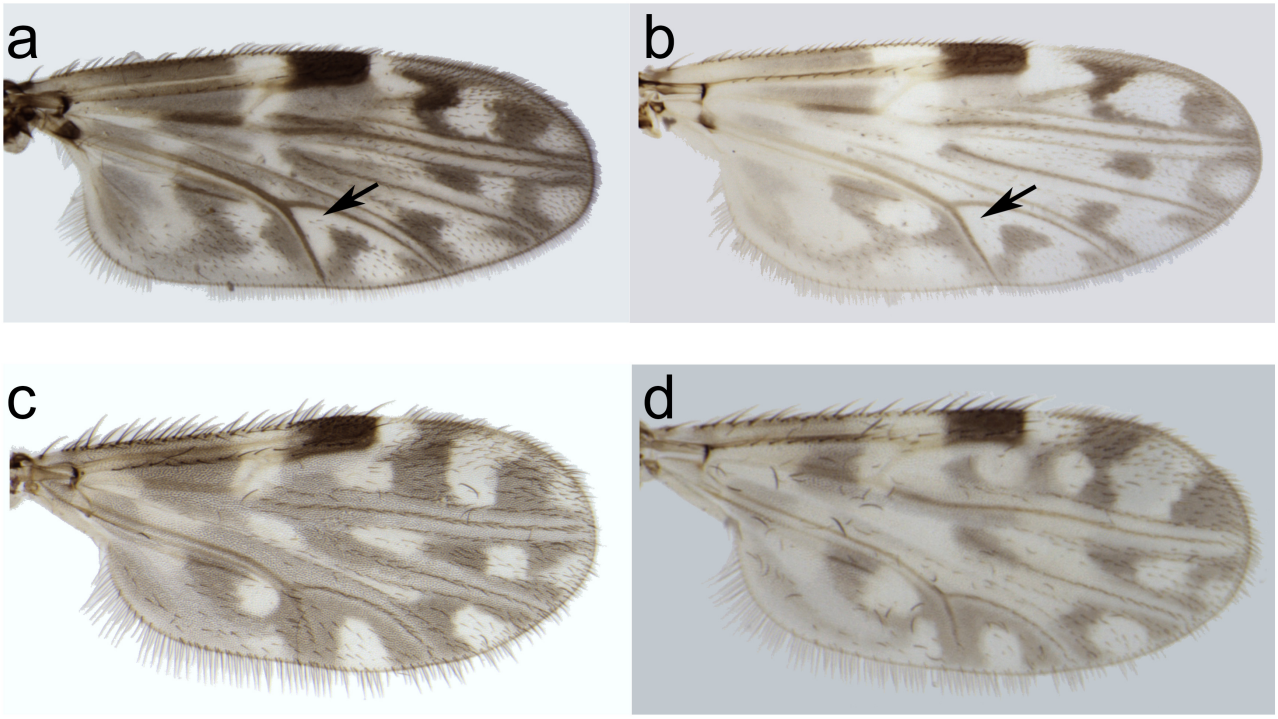

FIGURE 2. (a–d). Wings of *C. variipennis* (a), *C. sonorensis* (b), *C. stellifer* (c), *C. furens* (d)

3 (2). Basal 0.25 of wing pale, with isolated dark spot basal to R-M crossvein (Fig. 3-a) . . . . . *C. loughnani*

Basal 0.25 of wing with more extensive dark markings basal to R-M crossvein (Fig. 3, b–c) 4

FIGURE 3. (a–c). Wings of *C. loughnani* (a), *C. variipennis* (b), *C. sonorensis* (c)

4 (3). Third palpal segment narrow, sensory pit small (Fig. 4-a); wing mostly dark (Fig. 4-b). . . . . *C. variipennis*

Third palpal segment widened with expanded sensory pit (Fig. 4-c); wing mostly pale (Fig. 4-d). . . . . *C. sonorensis*

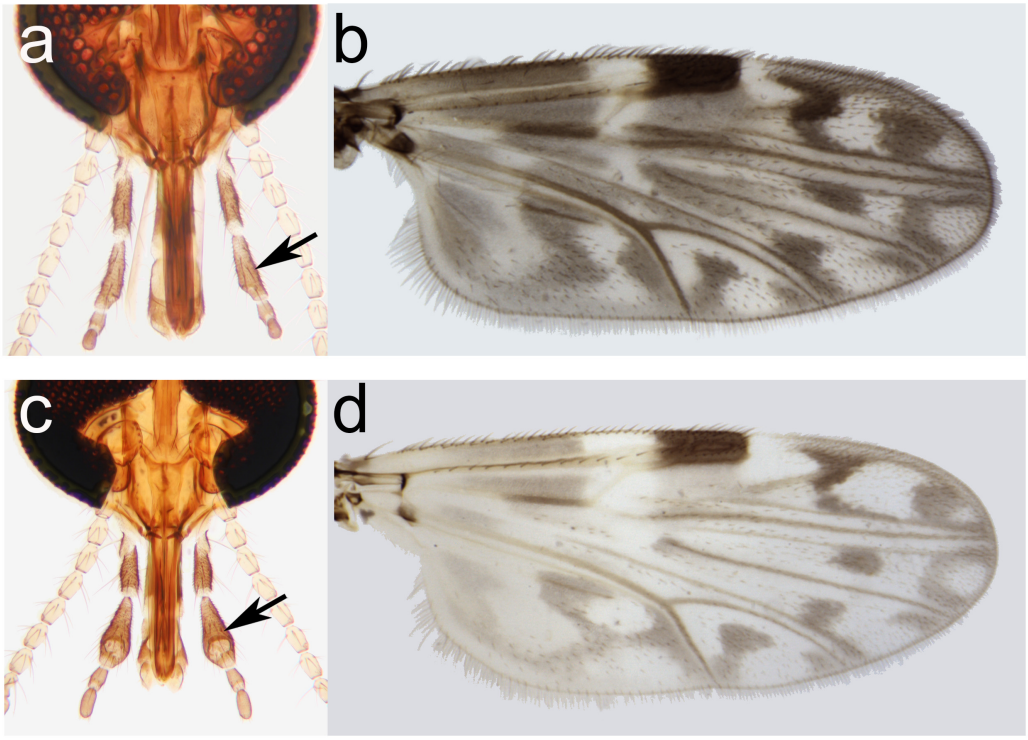

FIGURE 4. (a–d). Head and wing of *C. variipennis* (a, b), *C. sonorensis* (c, d)

5 (2). Wing veins M_1 and M_2 with pale borders; cell r_3 dark at apex, with median pale spot small and round, distal pale spot large and near middle of cell (Fig. 5-a); scutum with punctations (Fig. 5-b) . . . . . *C. furens*

Wing veins M_1 and M_2 without pale borders (Fig. 5-c); cell r_3 with small pale spot at apex, median pale spot larger; scutum without punctations (Fig. 5-d) 6

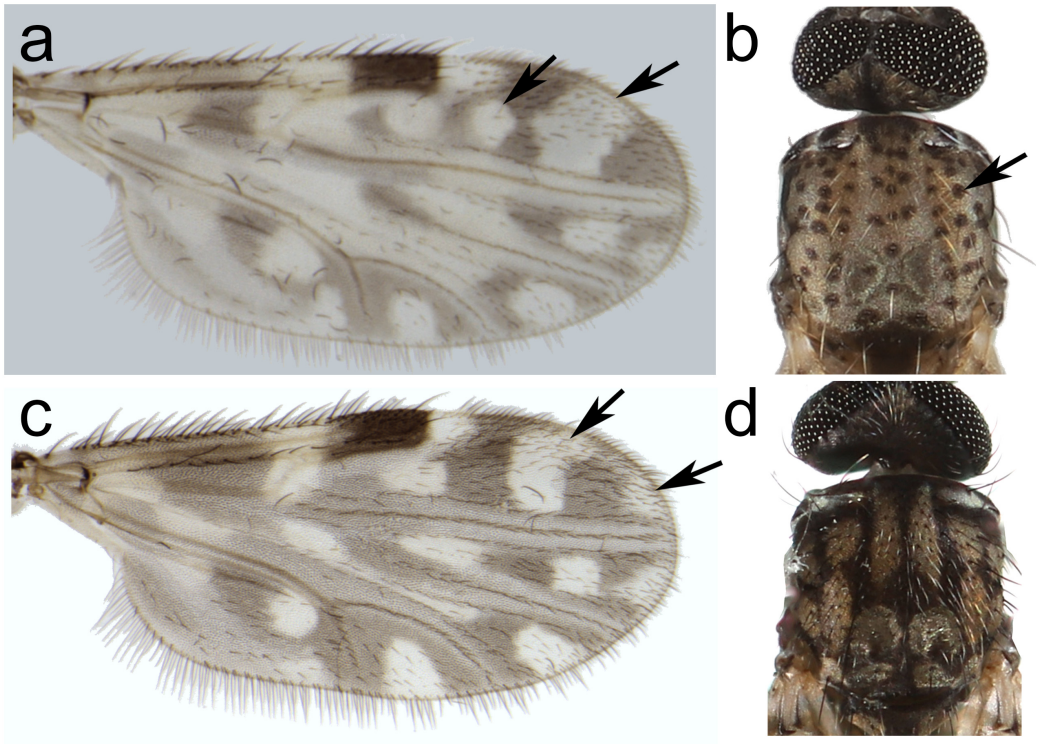

FIGURE 5. (a–d). Wing and scutum of *C. furens* (a, b), *C. stellifer* (c), and *C. arboricola* (d)

6 (5). Distal portion of anal cell with 2 spots; median spot in cell r_3 contacting wing margin (Fig. 6-a); hind tibia with narrow, median dark band covering less than one third (Fig. 6-b); third palpal segment with large, irregular sensory pit; larger species (Fig. 6-c) . . . . . *C. stellifer*

Distal portion of anal cell with 1 spot; median spot in cell r_3 not contacting wing margin (Fig. 6-d); hind tibia with broad, median dark band covering nearly half (Fig. 6-e); third palpal segment with small, deep sensory pit; smaller species (Fig. 6-f) . . . . . *C. paraensis*

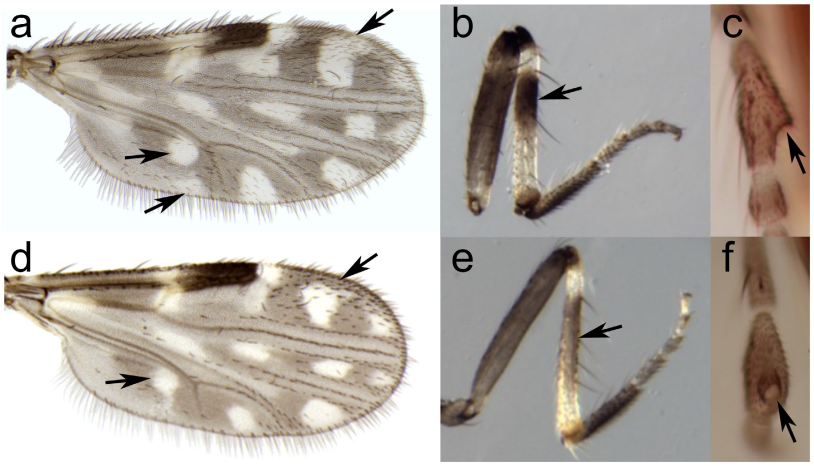

FIGURE 6 (a–f). Wings, hind leg and palpus of *C. stellifer* (a, b, c), and *C. paraensis* (d, e, f)

7 (1). Cell r_3 with apical pale spot midway between poststigmatic pale spot and apex of cell, apical 0.20–0.33 of cell dark (Fig. 7, a–d) 8

Cell r_3 without pale spot midway between poststigmatic pale spot and apex of cell, apical 0.20–0.33 of cell dark (Fig. 7-e), or with pale spot (Fig. 7, f–h) 22

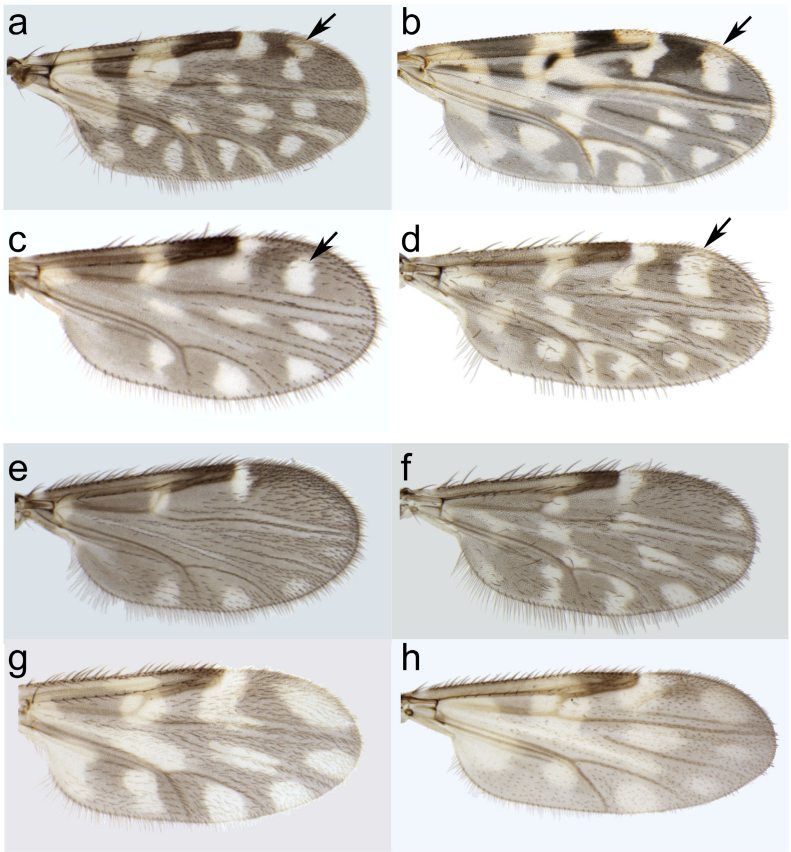

FIGURE 7 (a–h). Wings of *C. arboricola* (a), *C. insignis* (b), *C. debilipalpis* (c), *C. baueri* (d), *C. nanus* (e), *C. haematopotus* (f), *C. bickleyi* (g), and *C. hollensis* (h)

8 (7). Second radial cell mostly within a large pale spot (Fig. 8, a–b). 9

Second radial cell dark (Fig. 8, c–d). 10

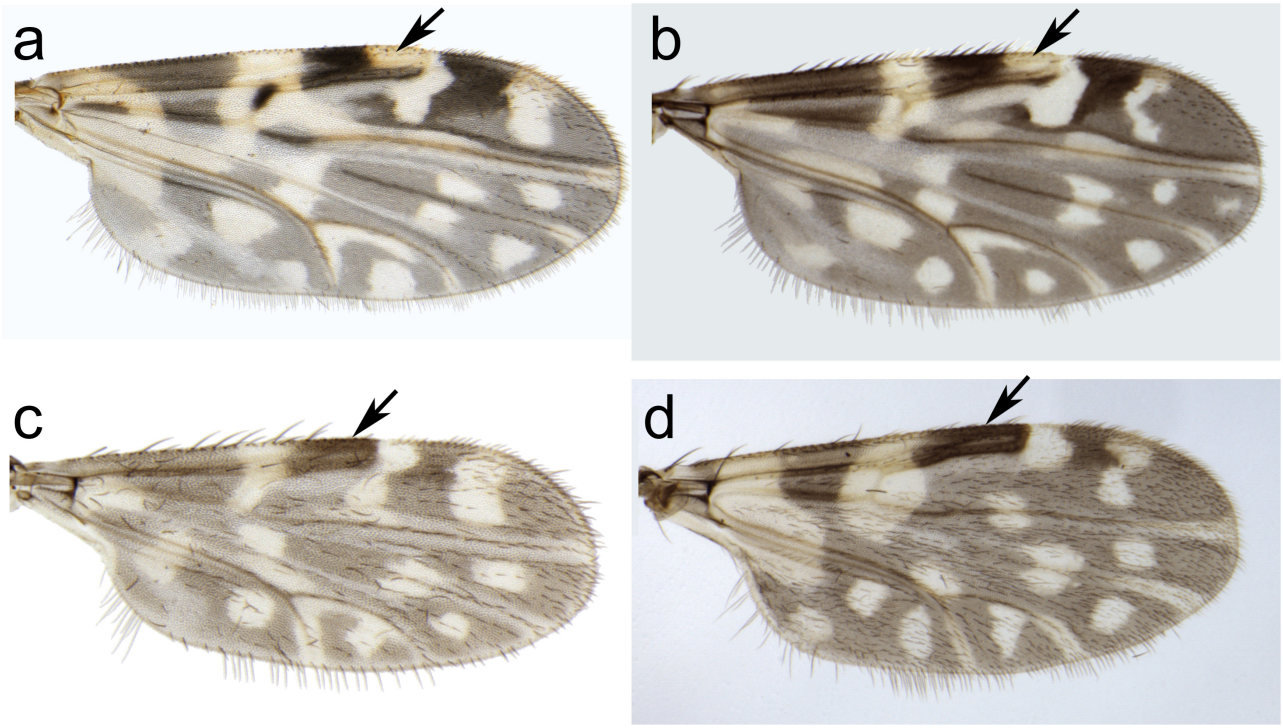

FIGURE 8 (a–d). Wings of *C. insignis* (a), *C. venustus* (b), *C. baueri* (c), *C. arboricola* (d)

9 (8). R-M crossvein with an isolated dark spur-shaped spot (Fig. 9-a) . . . . . .*C. insignis*

R-M crossvein without an isolated dark spur (Fig. 9-b). . . . . *C. venustus*

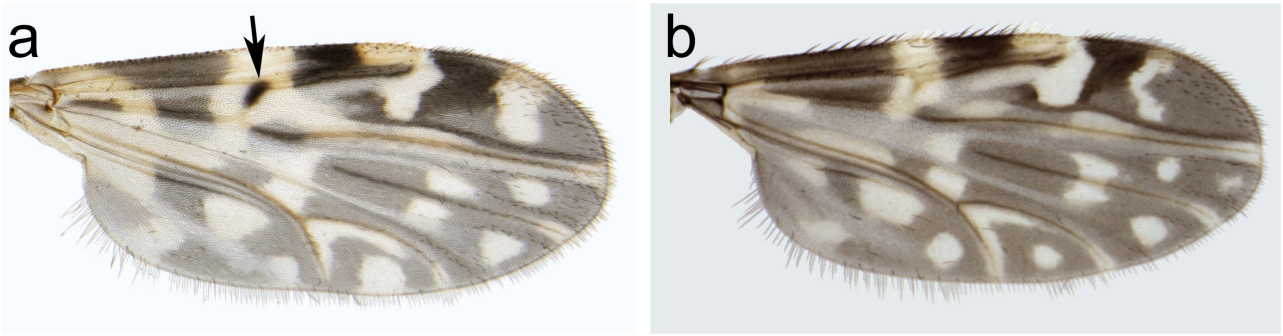

FIGURE 9 (a–b). Wings of *C. insignis* (a), and *C. venustus* (b)

10 (8). Apical pale spot of cell r_3 very narrow and curved (sigmoid or hourglass-shaped); anal cell with three pale spots in addition to any pale area at the base of the cell (Fig. 10, a–b). 11

Apical pale spot of cell r_3 usually broad, and rectangular (Fig. 10-c) or rounded (Fig. 10-d); anal cell with one or two pale spots (Fig. 10, c–d) 16

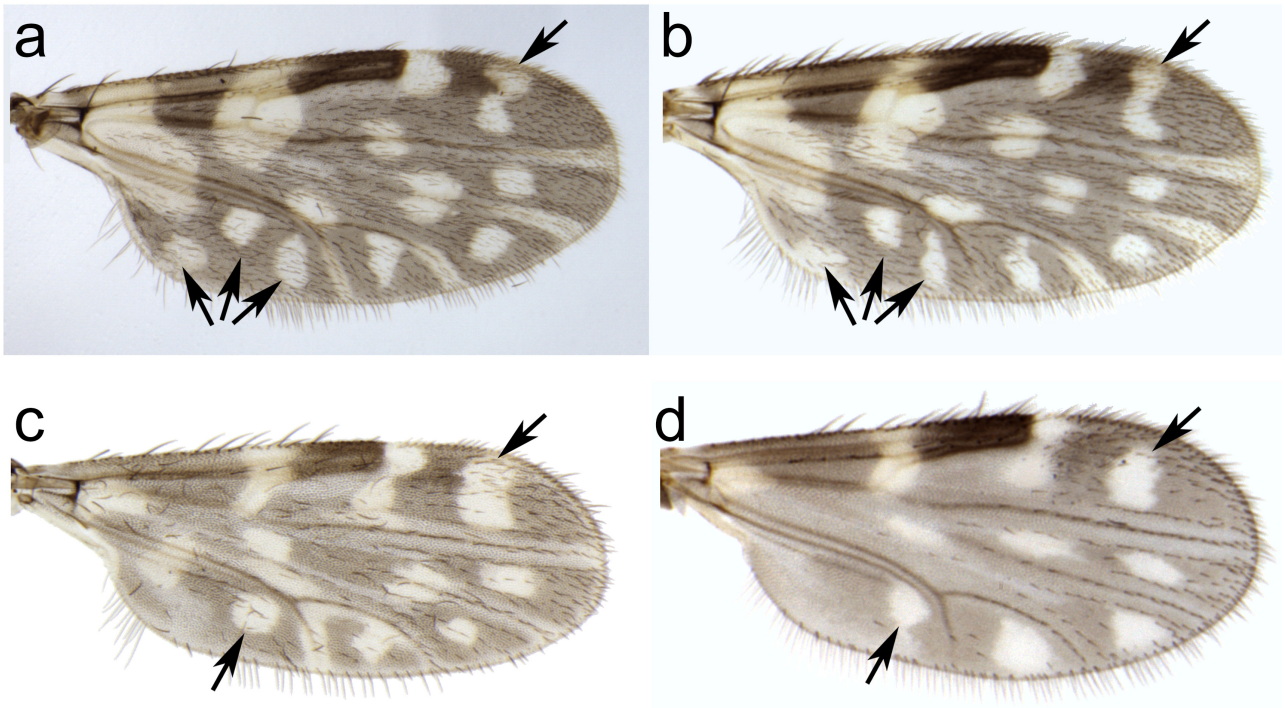

FIGURE 10. (a–d). Wings of *C. arboricola* (a), *C. guttipennis* (b), *C. baueri* (c), *C. debilipalpis* (d).

11 (10). Cell m_2 with distinct pale spot located directly below R-M crossvein (Fig. 11, a–b). 12

Cell m_2 without large pale spot area below R-M crossvein (Fig. 11, c–d). 14

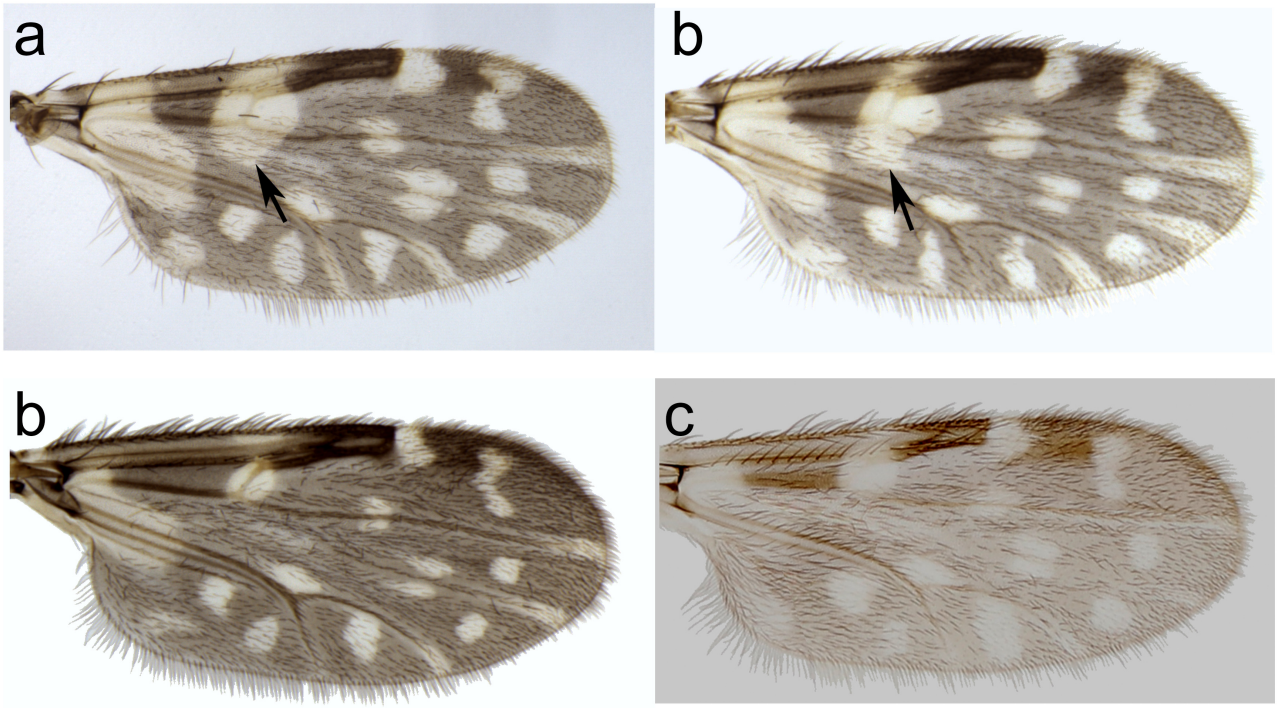

FIGURE 11. (a–d). Wings of *C. arboricola* (a), *C. guttipennis* (b), *C. beckae* (c), *C. ousairani* (d)

12 (11). Veins Cu_1 and Cu_2 both with pale borders (Fig. 12-a). . . . . *C. arboricola*

Vein Cu_1 with pale border, Cu_2 dark (Figs. 12 b–c) 13

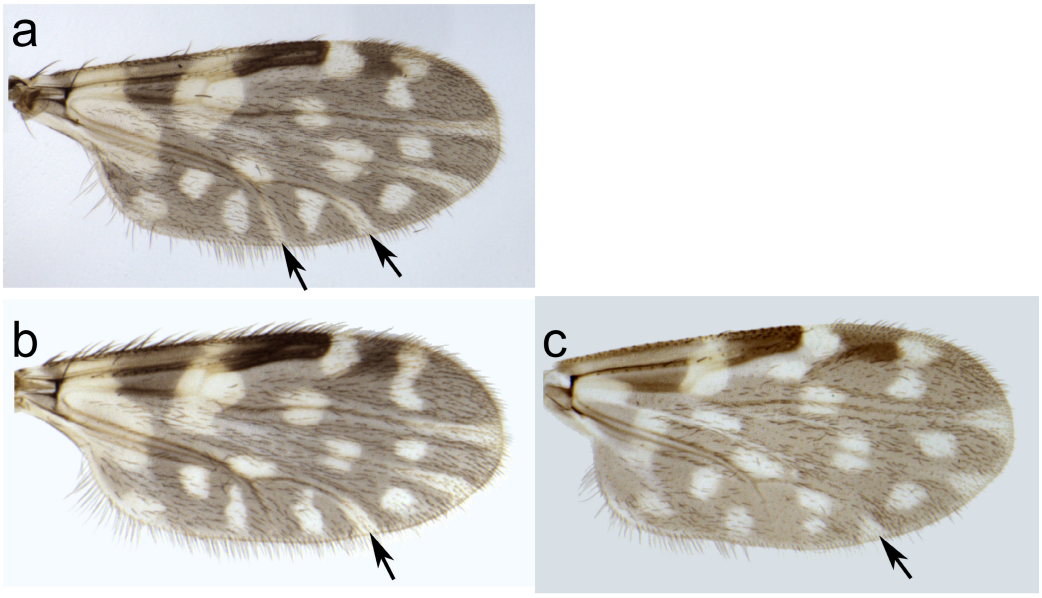

FIGURE 12. (a–c). Wings of *C. arboricola* (a), *C. guttipennis* (b), *C. jamaicensis* (c)

13 (12). Pale spots of cell m_2 and over R-M crossvein separated by vein M_1 (Fig. 13-a); third palpal segment greatly enlarged (Fig. 13-b). . . . . *C. jamaicensis*

Pale spots of cell m_2 and over R-M crossvein conjoined, forming large pale area (Fig. 13-c); third palpal segment not greatly enlarged (Fig. 13-d) . . . . . *C. guttipennis*

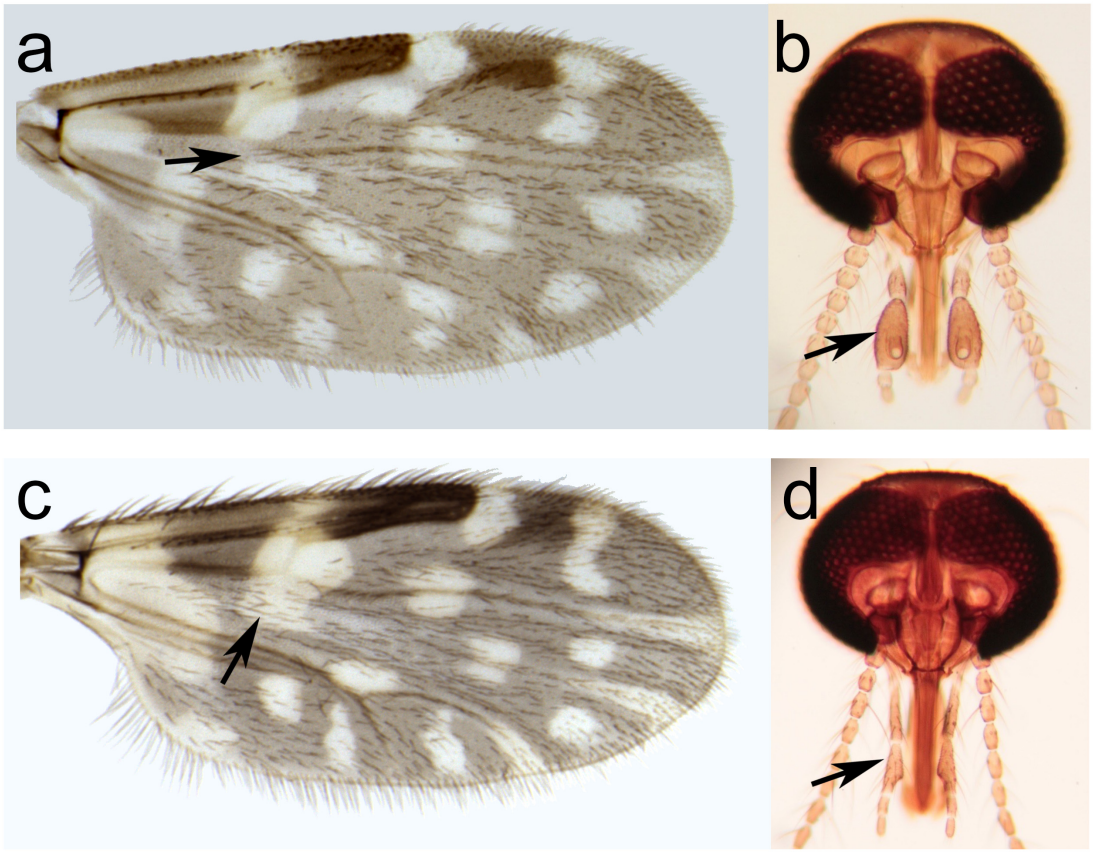

FIGURE 13. (a–d). Wing and head of *C. jamaicensis* (a, b), *C. guttipennis* (c, d)

14 (11). Distal five segments of antennae all greatly elongated, at least three times longer than proximal segments (Fig. 14-a). . . . . *C. villosipennis*

Distal five segments of antennae not all greatly elongated (Fig. 14-b) 15

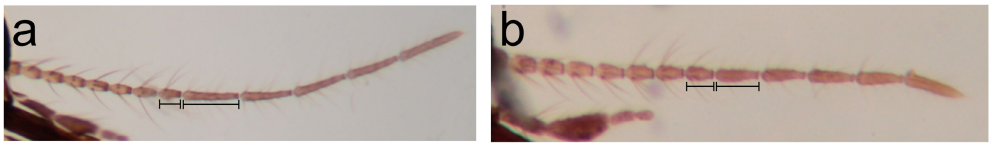

FIGURE 14 (a–b). Antenna of *C. villosipennis* (a), *C. ousairani* (b)

15 (14). Hind femur with strong subapical pale band (Fig. 15-a); third palpal segment moderately swollen with large sensory pit (Fig. 15-b); pale spot over R-M crossvein small (Fig. 15-c) . . . . . *C. beckae*

Hind femur with faint subapical pale band (Fig. 15-d); third palpal segment greatly swollen with small sensory pit (Fig. 15-e); pale spot over R-M crossvein larger (Fig. 15-f) . . . . . *C. ousairani*

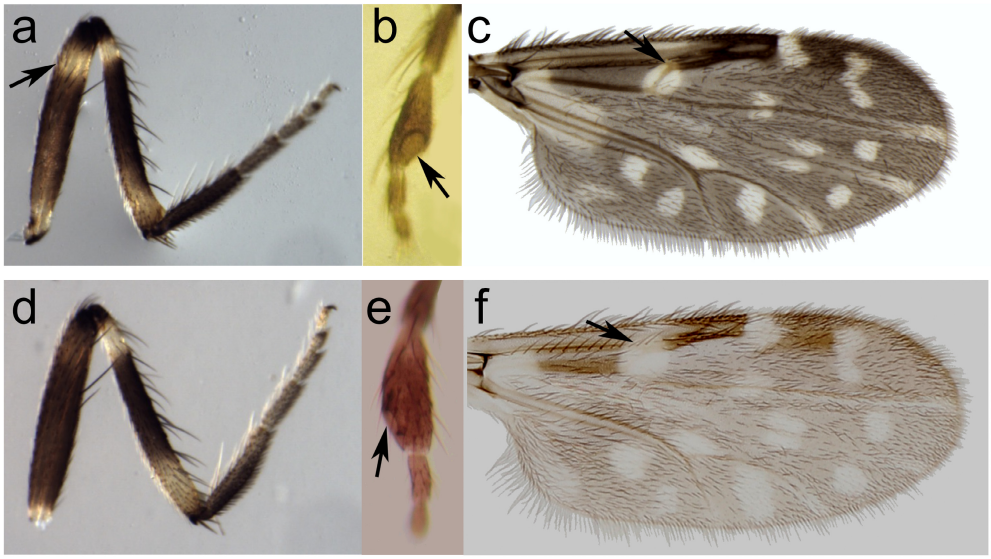

FIGURE 15 (a–f). Leg, palpus and wing of *C. beckae* (a–c), *C. ousairani* (d–f)

16 (10). Macrotrichia dense over nearly entire wing; cells m_1 and m_2 each with large, elongate, oval-shaped pale spot below poststigmatic pale spot (Fig. 16-a, b); one spermatheca present. 17

Macrotrichia sparse, especially on basal half of wing; pale spots below poststigmatic pale spot in cells m_1 and m_2, when present, small (Fig. 16-c), streak-like (Fig. 16-c), or straddling vein M_2 (Fig. 16-d), (not large, elongate, oval-shaped). 18

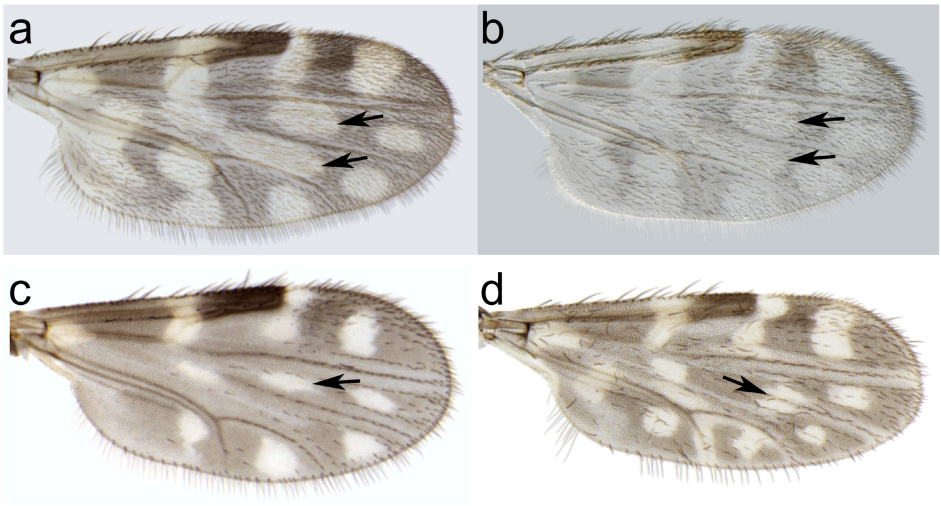

FIGURE 16 (a–d). Wings of *C. crepuscularis* (a), *C. knowltoni* (b), *C. debilipalpis* (c), *C. baueri* (d)

17 (16). Spermatheca long and narrow, 3.5–4.0 times longer than wide (Fig. 17-a, b) . . . . . *C. knowltoni*

Spermatheca oval, approximately twice as long as wide (Fig. 17-c) . . . . . *C. crepuscularis*

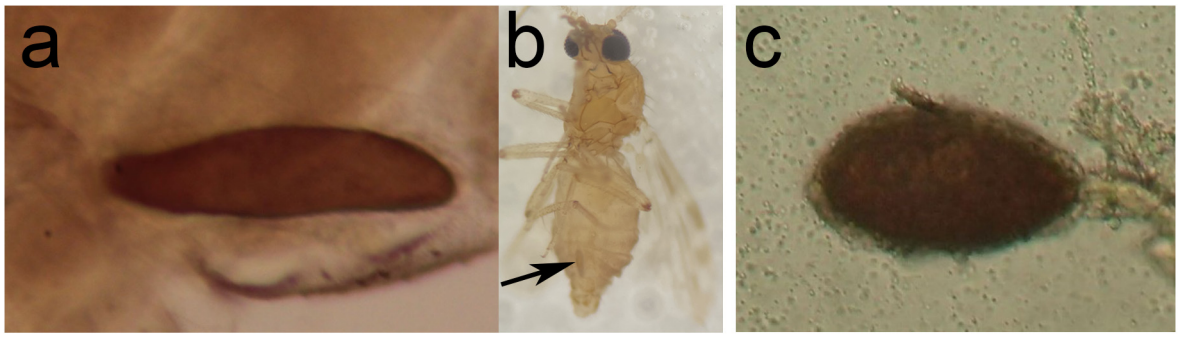

FIGURE 17 (a–c). Spermatheca of *C. knowltoni* (a, b), and *C. crepuscularis* (c)

18 (16). Vein Cu_2 pale at wing margin; apical pale spot in cell r_3 rectangular (Fig. 18-a) . . . . . *C. baueri*

Vein Cu_2 dark at wing margin; apical pale spot in cell r_3 rounded (Fig. 18-b). 19

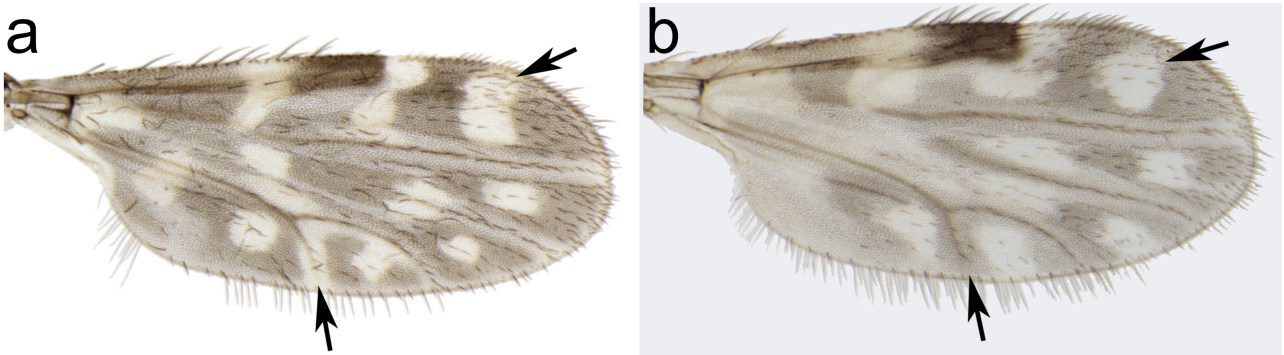

FIGURE 18 (a–b). Wings of *C. baueri* (a), and *C. barbosai* (b)

19 (18). Cells m_1 and m_2 each with streak-like pale spot between poststigmatic pale spot and anal cell, together forming a large spot that straddles vein M_2; apical pale spot in cell r_3 broadly reaching wing margin; anal cell with several (>10) macrotrichia (Fig. 19-a). . . . . *C. barbosai*

Cell m_1 with small pale spot between poststigmatic pale spot and anal cell; corresponding pale spot in cell m_2 usually absent, occasionally straddling M_2 (some *C. hinmani*); apical pale spot in cell r_3 not meeting wing margin; anal cell with few (0–5) macrotrichia (Fig. 19-b). 20

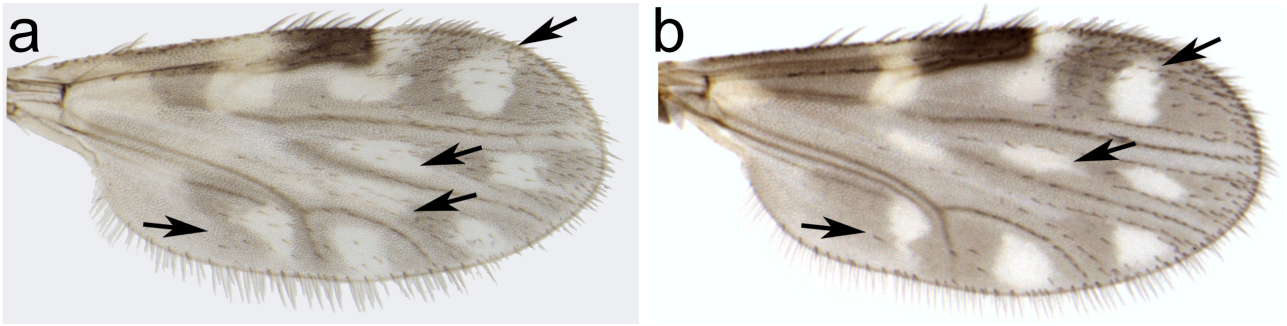

FIGURE 19 (a–b). Wings of *C. barbosai* (a), and *C. debilipalpis* (b)

20 (19). Wing cell r_3 with distal pale spot located nearer to wing apex than to poststigmatic spot (Fig. 20-a); pleuron yellowish brown with distinctive dark brown median spot (Fig. 20-b) . . . . . *C. hinmani*

Wing cell r_3 with distal pale spot located nearer to poststigmatic spot than to wing apex (Fig. 20-c); pleuron mainly dark (Fig. 20-d). 21

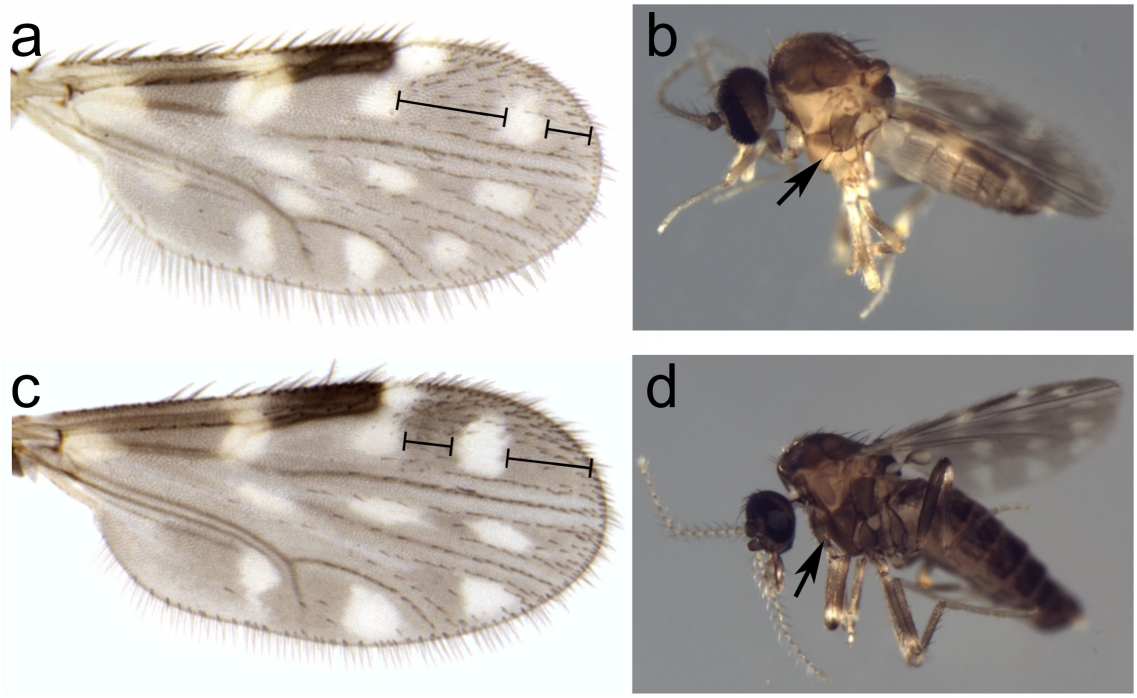

FIGURE 20 (a–d). Wing and body of *C. hinmani* (a, b), *C. debilipalpis* (c, d)

21 (20). Distal pale spots of cells r_3, m_1 and m_2 forming a straight line (Fig. 21-a); hind tibia with narrow subapical pale band, covering less than 0.25 (Fig. 21-b); third palpal segment with small, deep sensory pit (Fig. 21-c) . . . . . *C. debilipalpis*

Distal pale spot of cell m_1 out of line with spots in cells r_3 and m_2 (Fig. 21-d); hind tibia with wide subapical pale band covering at least 0.33 (Fig. 21-e); third palpal segment with large, shallow sensory pit (Fig. 21-f) . . . . . *C. torreyae*

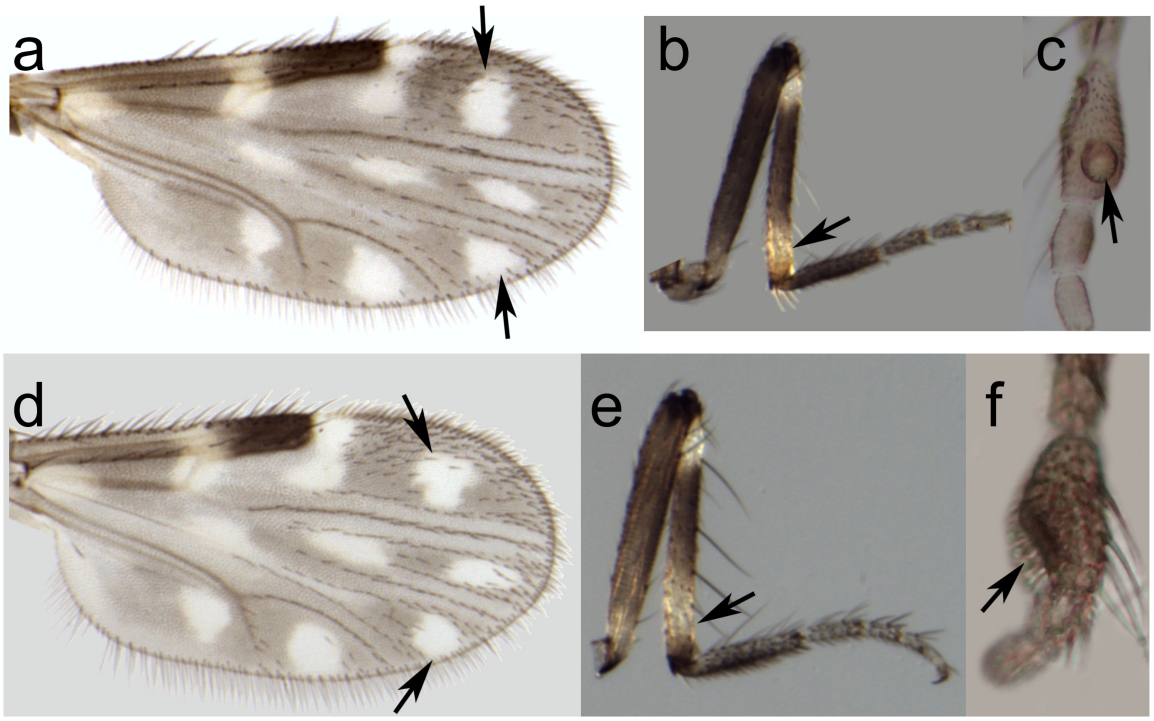

FIGURE 21 (a–f). Wing, hind leg, and palp of *C. debilipalpis* (a–c), *C. torreyae* (d–f)

22 (7). Thorax yellowish (Fig. 22-a); wing yellowish gray without pattern of pale spots (Fig. 22-b) 23

Thorax variable, not yellowish (Fig. 22-c); wing not yellowish, with at least one pale spot, over R-M crossvein or at apical end of second radial cell; if no pale spots, then wing very pale (Fig. 22-d) 24

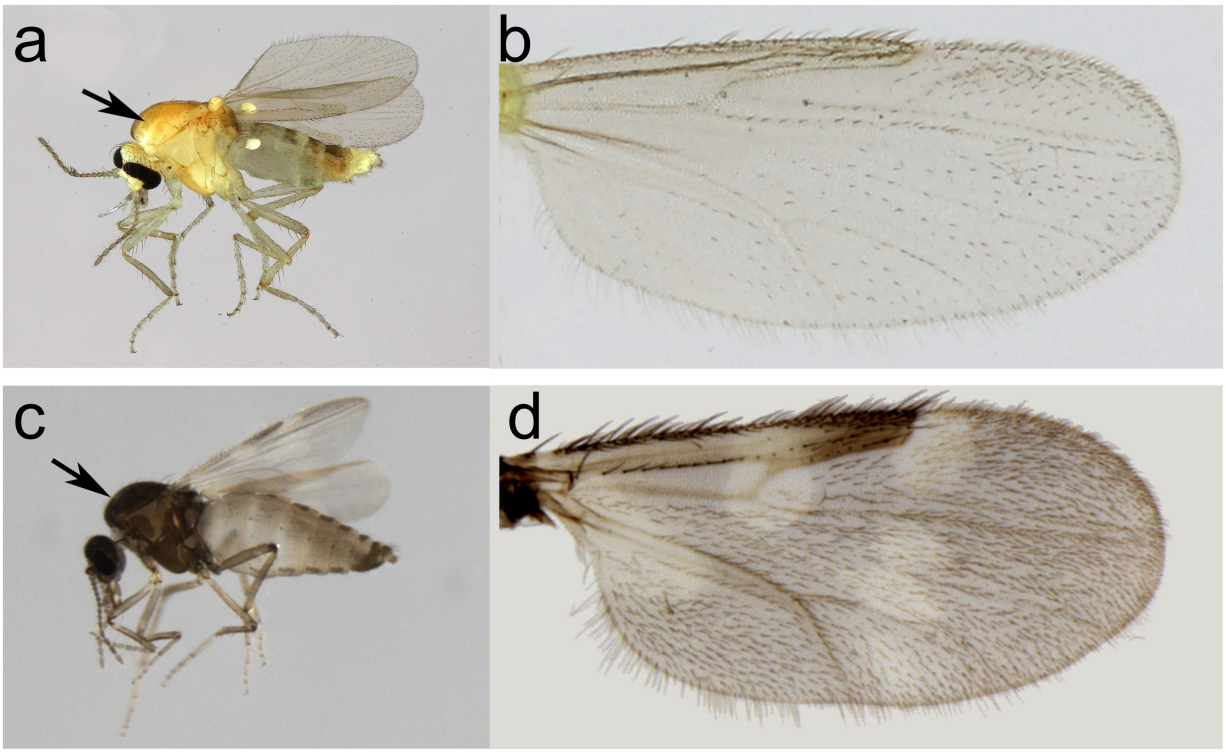

FIGURE 22 (a–d). Body and wing of *C. melleus* (a, b), *C. pallidicornis* (c, d)

23 (22). Thorax dorsum pale yellow (Fig. 23-a); wing with very few macrotrichia (Fig. 23-b); spermathecae yellow (Fig. 23-c); small, freshwater species. . . . . *C. floridensis*

Thorax dorsum darker shade (Fig. 23-d); wing with numerous macrotrichia (Fig. 23-e); spermathecae dark brown (Fig. 23-f); larger, coastal species. . . . . *C. melleus*

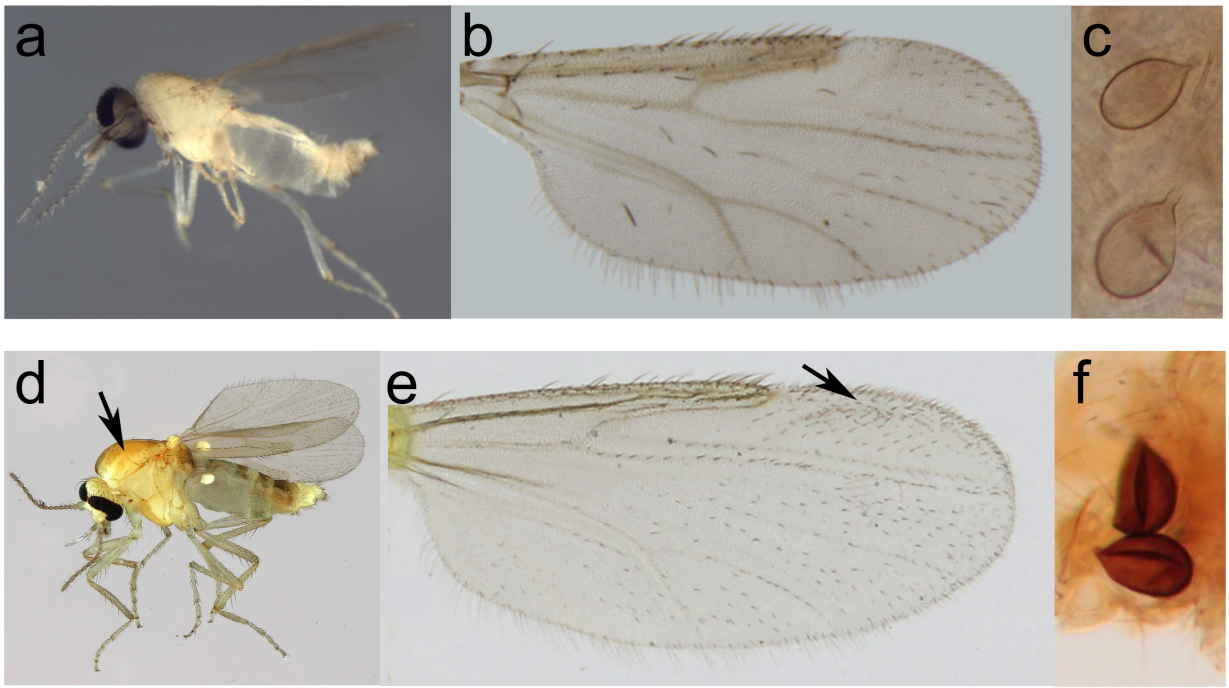

FIGURE 23 (a–f). Body, wing and spermatheca of *C. floridensis* (a–c), *C. melleus* (d–f)

24 (22). Eyes contiguous (Fig. 24-a); macrotrichia absent, or restricted to distal third of wing, mainly along veins, absent in anal cell and cua_1 (Fig. 24-b) 25

Eyes narrowly to broadly separated (Fig. 24-c); macrotrichia present on most of wing, present in anal cell and cua_1 (Fig. 24-d). 29

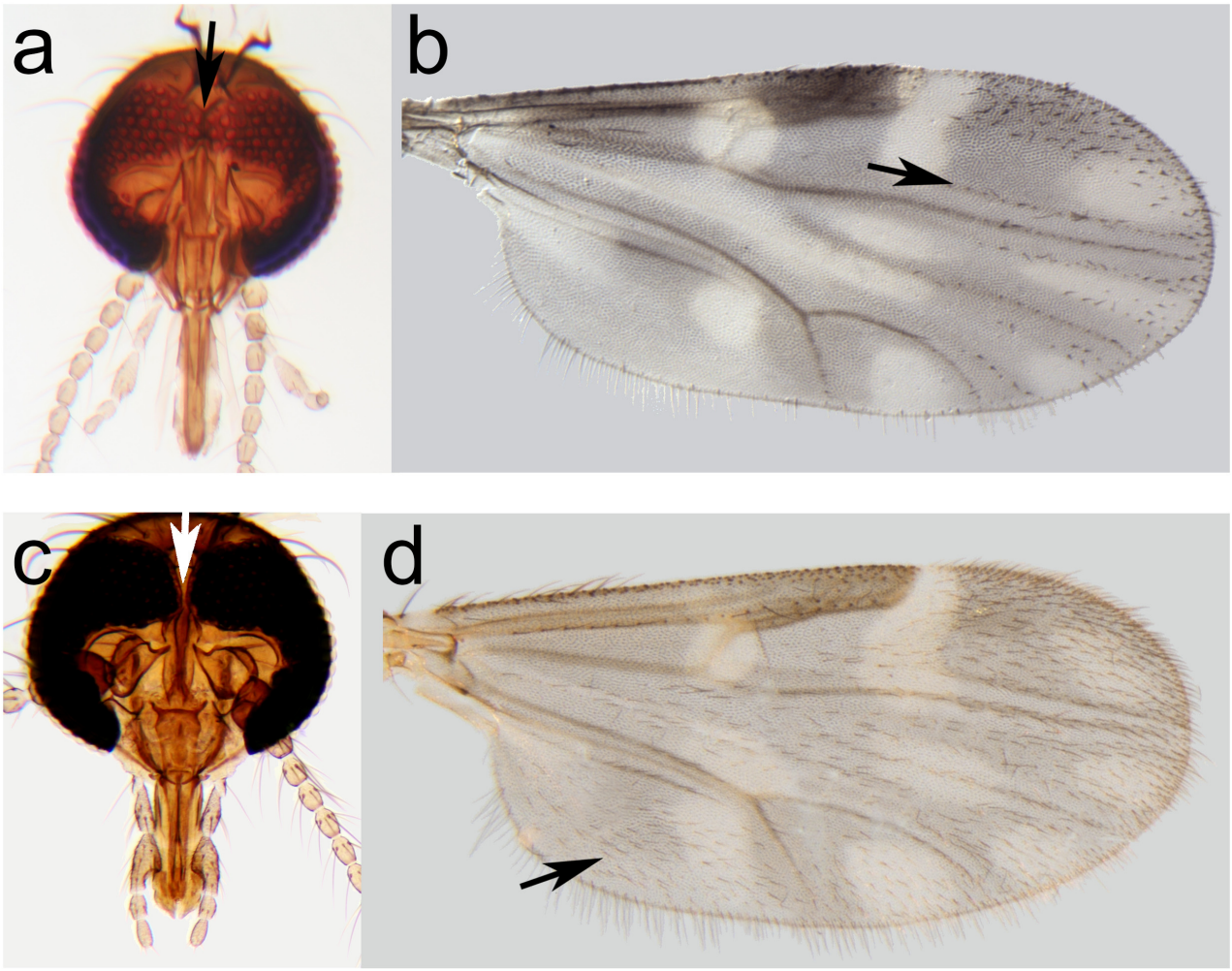

FIGURE 24 (a–d). Head and wing of *C. chiopterus* (a), *C. alachua* (b), *C. bickleyi* (c) *and C. scanloni* (d)

25 (24). Wing cells r_3 m_1 and m_2 each with distinct pale spot reaching wing margin; pale spot over R-M crossvein small and rounded, not reaching costa or into cell m_2 (Fig. 25-a). . . . . *C. alachua*

Wing cells r_3 m_1 and m_2 without distinct pale spots; pale spot over R-M crossvein large and elongate, usually reaching costa and/or into cell m_2 (Fig. 25-b). 26

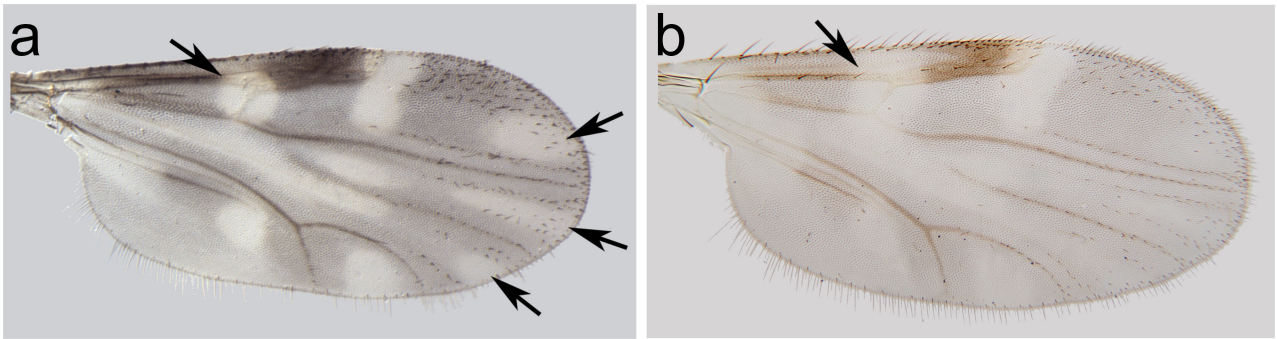

FIGURE 25 (a–b). Wing of *C. alachua* (a), and *C. chiopterus* (b)

26 (25). Wing without macrotrichia (Fig. 26-a); thorax blackish (Fig. 26-b); proboscis very long (P/H ratio 1.08, Fig. 26-c). . . . . *C. pusillus*

Wing with several to few macrotrichia on membrane (Fig. 26-d); thorax variable; proboscis not so long (P/H ratio <1.0, Fig. 26-e).. 27

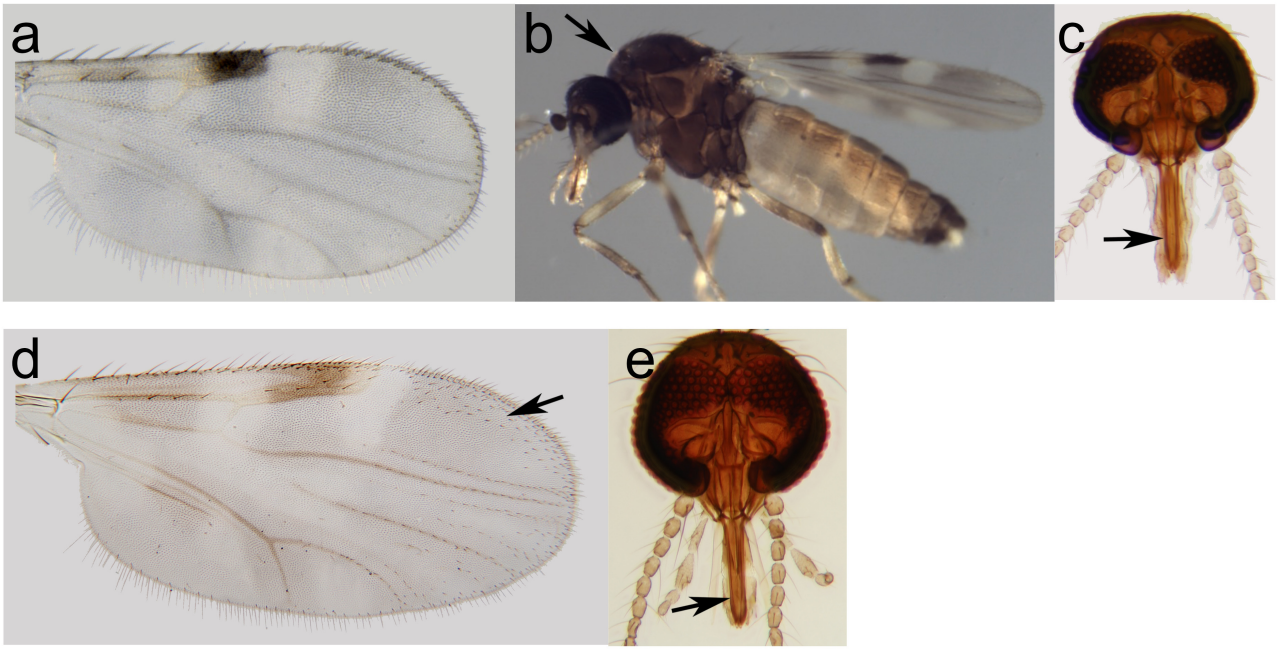

FIGURE 26 (a–e). Wing, body and head of *C. pusillus* (a–c), and *C. chiopterus* (d–e)

27 (26). Wing with macrotrichia restricted to few at apices of cells r_3 and m_1 (Fig. 27-a); proboscis very short (P/H ratio 0.44, Fig. 27-b); very rare in Florida, probably does not bite . . . . . *C. pechumani*

Wing with sparse macrotrichia on distal third of wing, including cells r_3, m_1 and m_2 (Fig. 27-c); proboscis longer (P/H ratio >0.5, Fig. 27 d–e). 28

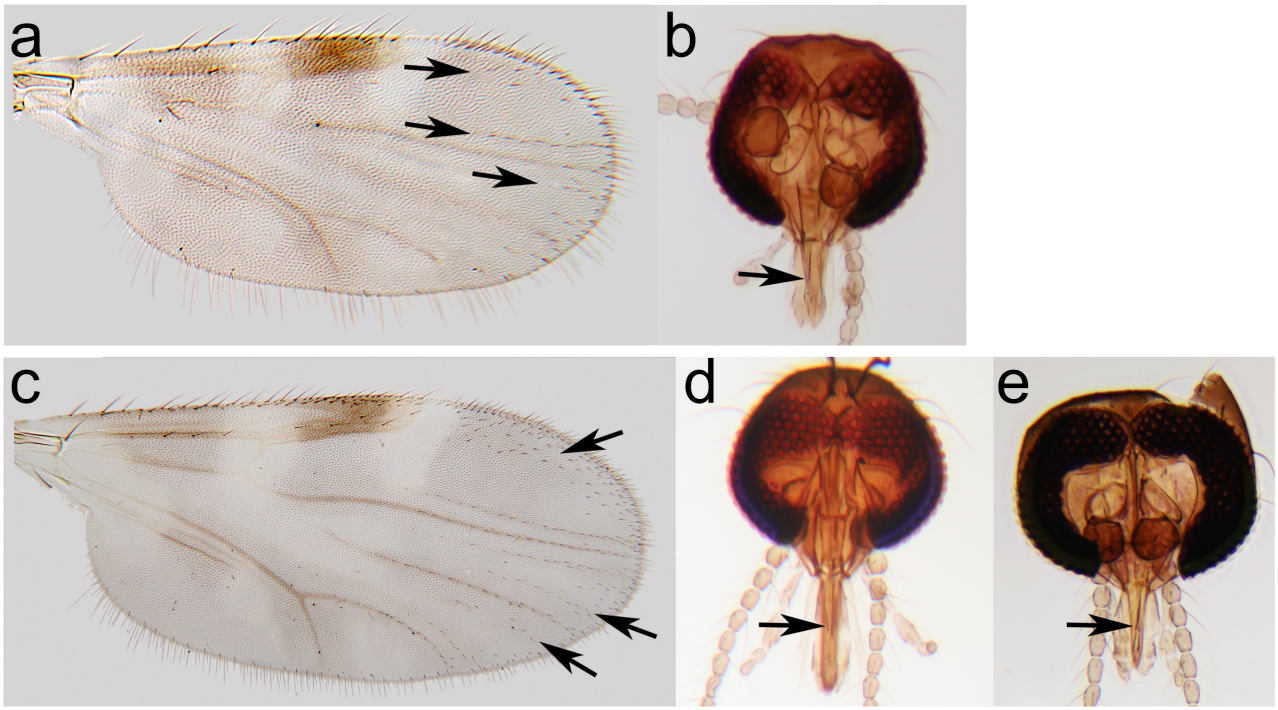

FIGURE 27 (a–e). Wing of and head *C. pechumani* (a–b), *C. chiopterus* (c,d) and *C. juddi* (e)

28 (27). Distal 0.33 of 2nd radial cell within pale spot (Fig. 28-a); proboscis short (P/H ratio 0.60, Fig. 28-b); lacking tormae and teeth on mandible; very rare in Florida (1 record, Jefferson County). . . . . *C. juddi*

Distal 0.50 of 2nd radial cell within pale spot (Fig. 28-c); proboscis slightly longer (P/H ratio 0.66, Fig. 28-d); tormae and teeth present on mandible; rare in Florida. . . . . *C. chiopterus*

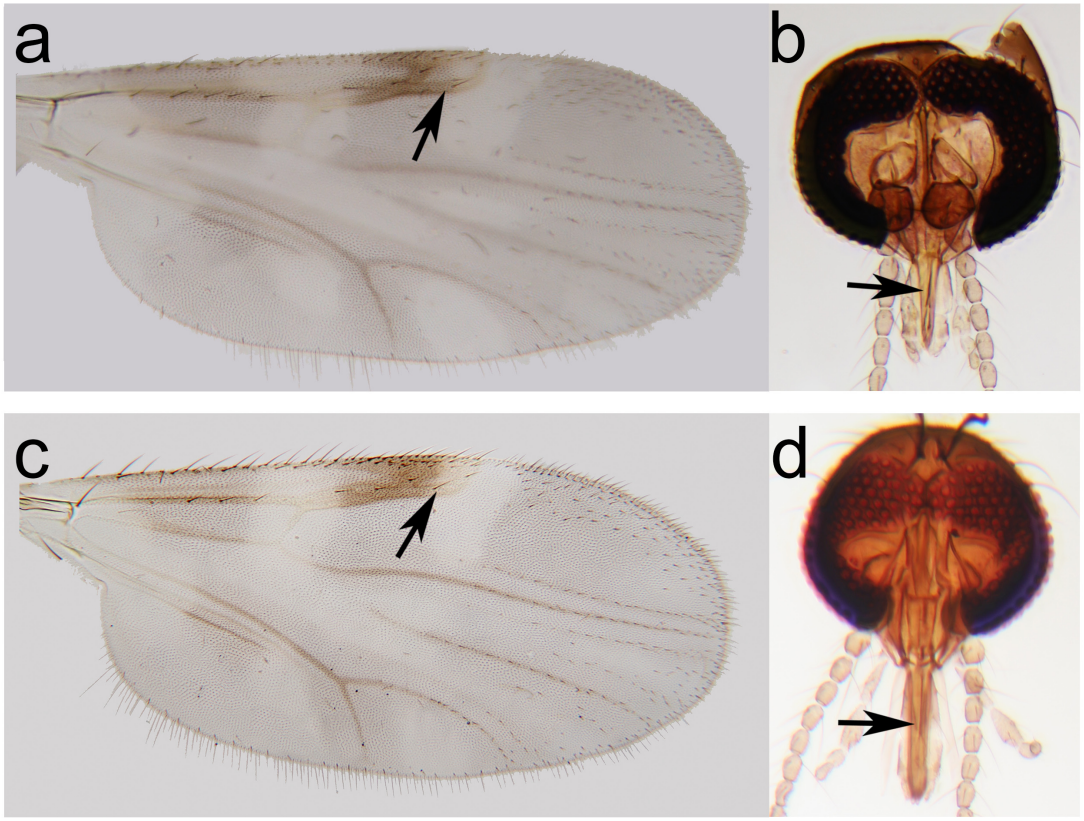

FIGURE 28 (a–d). Wing and head of *C. juddi* (a, b), and *C. chiopterus* (c, d)

29 (24) Wing cell r_3 with distinct apical pale spot reaching or approaching wing margin (Fig. 29 a, b) 30

Wing cell r_3 without pale spot in distal half of cell (Fig. 29 c), or with pale spot very faint or appearing as a pale streak between darker wing veins (Fig. 29 d). 36

FIGURE 29 (a–d). Wing of *C. haematopotus* (a), *C. mississippiensis* (b), *C. nanus* (c) and *C. travisi* (d)

30 (29). Apical pale spot of cell r_3 small, distinct, restricted to apical 0.25 of cell r_3 (Fig. 30-a,b) 31

Apical pale spot of cell r_3 large, typically covering at least apical 0.4 of cell beyond 2nd radial cell (Fig. 30-c, d). 32

FIGURE 30 (a–d). Wing of *C. haematopotus* (a), *C. edeni* (b), *C. mississippiensis* (c) and *C. bickleyi* (d)

31 (30). Thorax yellowish brown (Fig. 31-a); pale spot over R-M crossvein extending to costal margin; anal cell with strong proximal pale spot near wing margin (Fig. 31-b); southern Florida . . . . . *C. edeni*

Thorax dark brown with paler markings (Fig. 31-c); pale spot over R-M crossvein not extending to costal margin; anal cell without strong proximal pale spot near wing margin (Fig. 31-d); northern Florida. . . . . .*C. haematopotus*

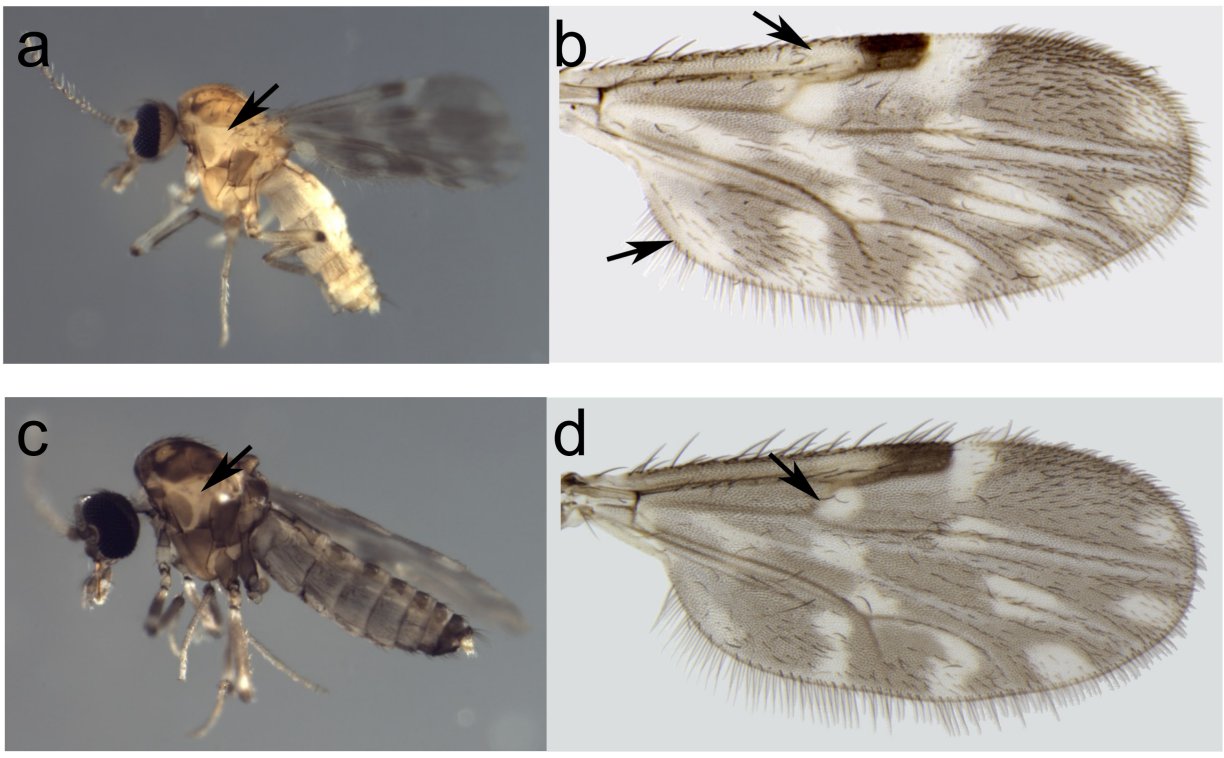

FIGURE 31 (a–d). Body and wing of *C. edeni* (a, b), and *C. haematopotus* (c, d)

32 (30). Cells m_1 and m_2 with elongate median pale spots in line with poststigmatic pale spot . . . . . .*C. mississippiensis/C. hollensis*

Cells m_1 and m_2 without median pale spots (Fig. 32 c, d) 33

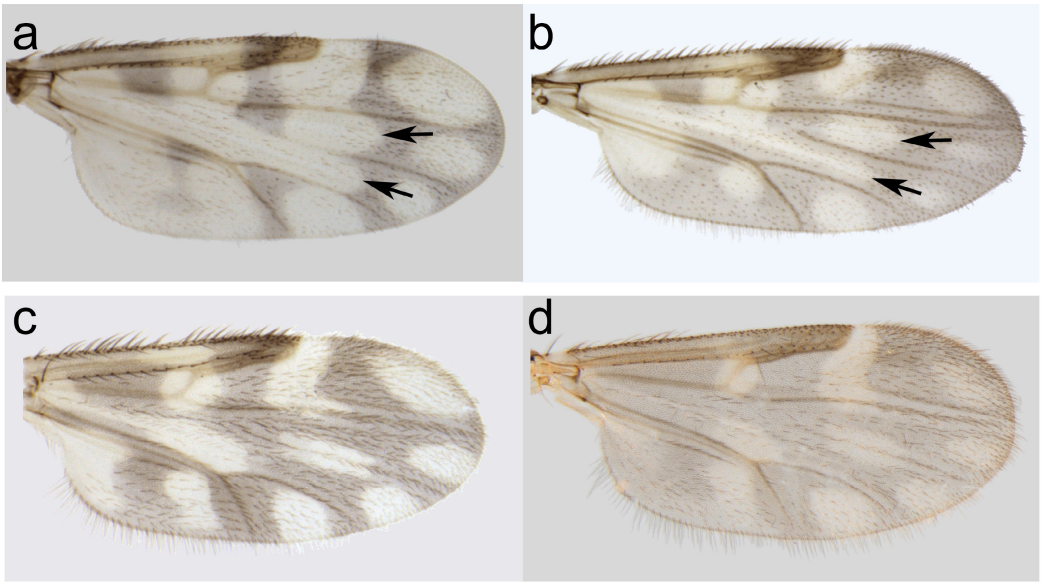

FIGURE 32 (a–d). Wing of *C. mississippiensis* (a), *C. hollensis* (b), *C. bickleyi* (c) and *C. scanloni* (d)

33 (32). Proximal antennal segments extremely short (about as wide as long), combined proximal segments about half the length of combined distal segments (AR 1.87; Fig. 33-a); third palpal segments greatly swollen and asymmetrical; proboscis moderately long (P/H ratio 0.71) (Fig. 33-b) . . . . . *C. piliferus*

Proximal antennal segments each longer than wide (Fig. 33-c), combined length only slightly shorter than length of combined distal segments (AR 1.20-1.47, Fig. 33-d); third palpal segments slightly to moderately swollen (Fig. 33-d); proboscis length variable. 34

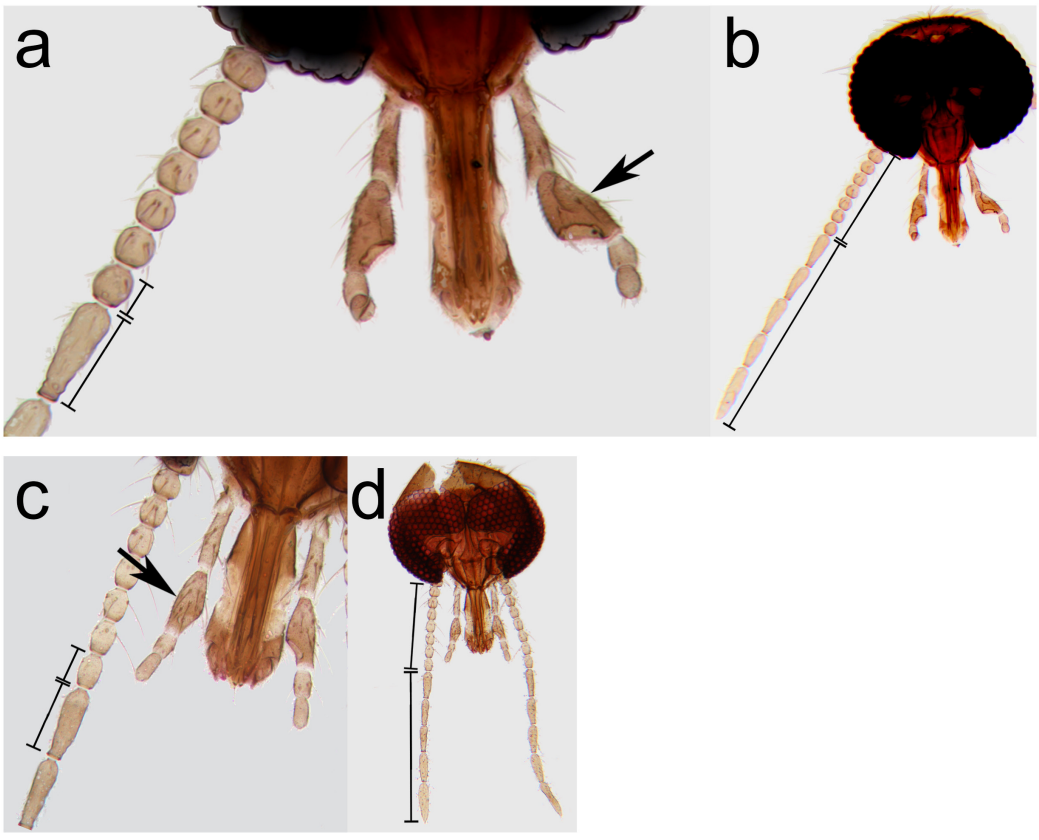

FIGURE 33 (a–d). Head of *C. piliferus* (a, b), and *C. scanloni* (c, d)

34 (33). Proboscis short (P/H ratio 0.62, Fig. 34-a); apex of 2nd radial cell occasionally pale (Fig. 34-b); rare in Florida, with few confirmed records. . . . . .*C. bickleyi*

Proboscis longer (P/H ratio 0.78–0.92, Fig. 34-c), moderately shorter than head; apex of 2nd radial cell rarely pale (Fig. 34-d). 35

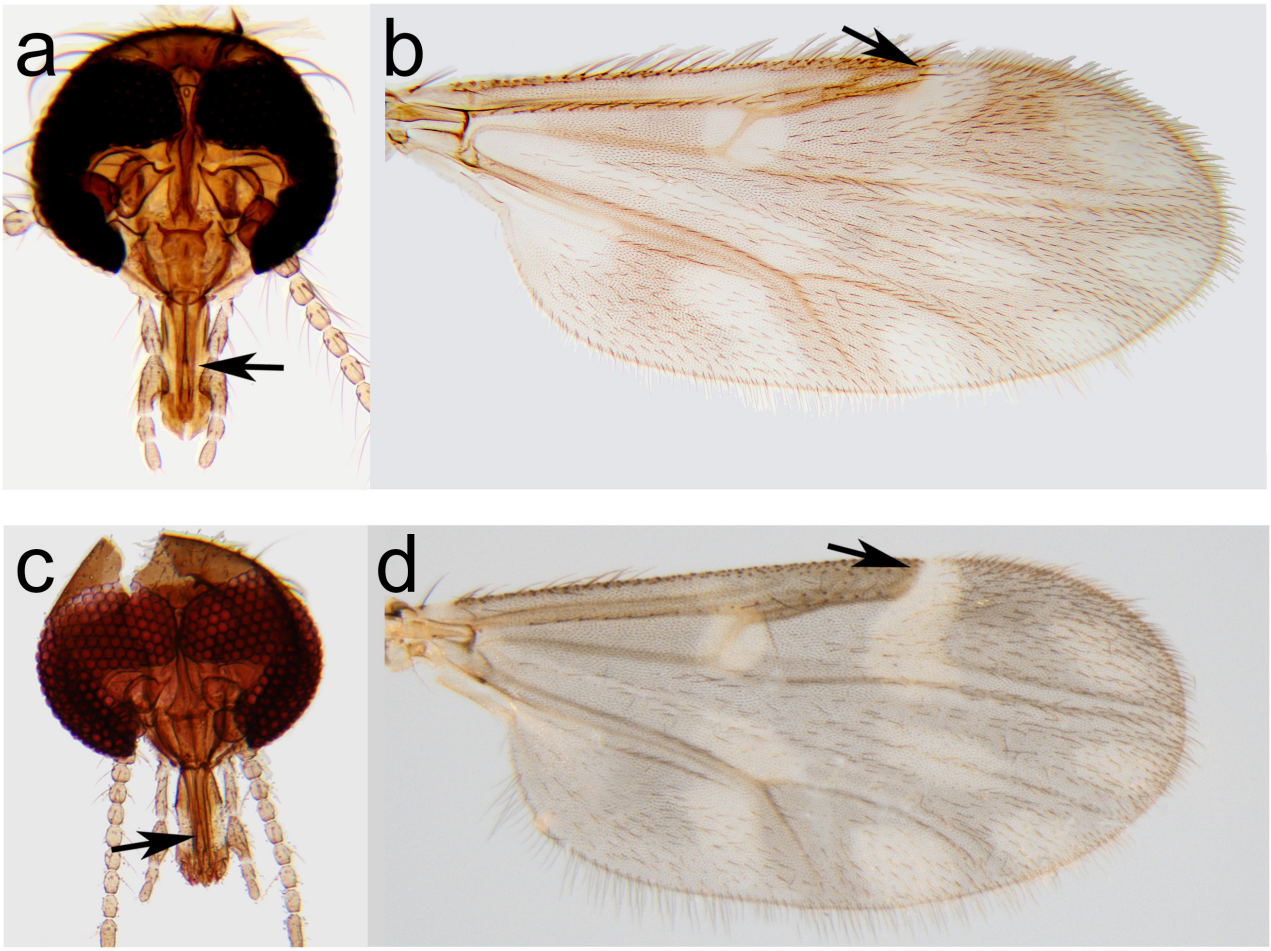

FIGURE 34 (a–d). Head and wing of *C. bickleyi* (a, b), and *C. scanloni* (c, d)

35 (34). Proboscis moderately long (P/H 0.78, Fig. 35-a); fore and mid femora with subapical pale band (Fig. 35-b); abdomen pale brown, thorax brown (Fig. 35-c); common in Florida . . . . . .*C. scanloni*

Proboscis very long (P/H 0.92); femora not banded; abdomen dark brown, thorax brownish black (not pictured); very rare in Florida: just 1 record, Jackson County. . . . . *C. parapiliferus*

FIGURE 35 (a–c). Head, leg and body of *C. scanloni* (a, b, c)

36 (29). Wing very pale, without distinct pale spots (Fig. 36-a, b). 37

Wing dark with distinct pale spots over R-M crossvein and poststigmatic area (Fig. 36-c, d) 38

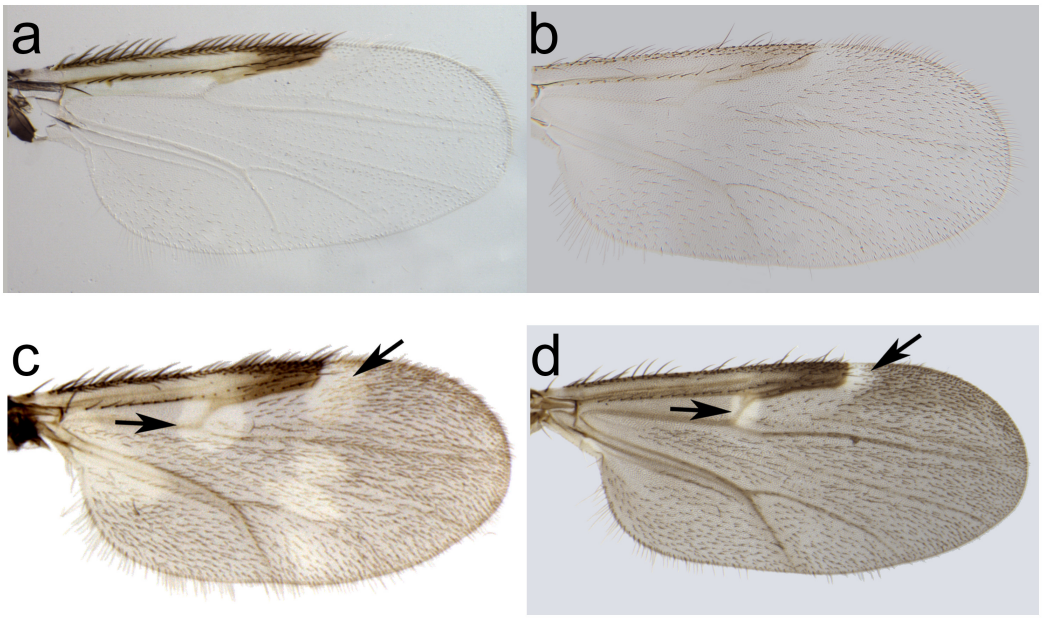

FIGURE 36 (a–d). Wing of *C. tissoti* (a), *C. snowi* (b), *C. pallidicornis* (c) and *C. biguttatus* (d)

37 (36). Wing membrane milky white, contrasting strongly with black anterior wing veins; strong black setae on costa (Fig. 37-a); proboscis long (P/H ratio 0.90; Fig. 37-b) . . . . . *C. tissoti*

Wing membrane creamy white, not contrasting strongly with second radial cell or anterior wing veins; costa lacking strong setae (Fig. 37-c); proboscis moderately long (P/H ratio 0.82, Fig. 37-d). . . . . *C. snowi*

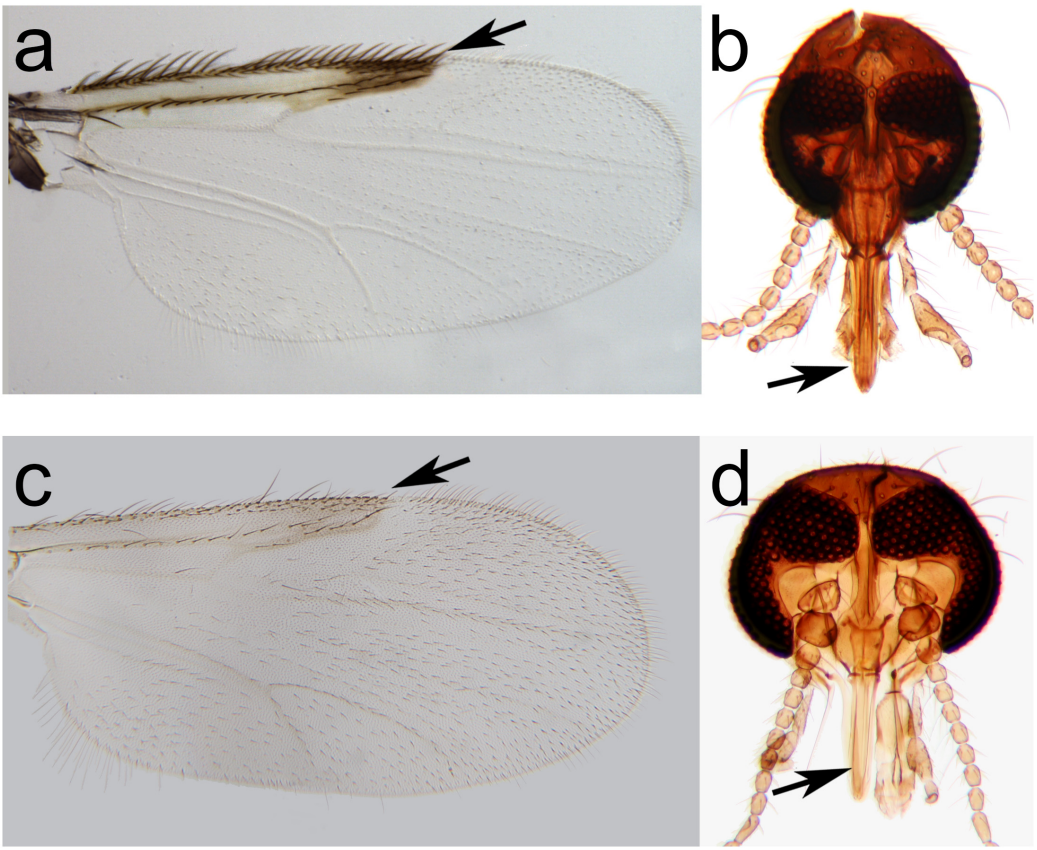

FIGURE 37 (a–d). Wing and head of *C. tissoti* (a, b), and *C. snowi* (c, d)

38 (36). Wing with transverse pale band near middle, forming isolated row of faint pale spots extending between the apex of the second radial cell and cell cua_1 (Fig. 38-a); thorax dark brown (Fig. 38-b); third palpal segment swollen apically with large shallow pit (Fig. 38-c) . . . . . *C. pallidicornis*

Wing without transverse pale band near middle (Fig. 38-d); thorax variable (Fig. 38-e); third palpal segment variable (Fig. 38-f) 39

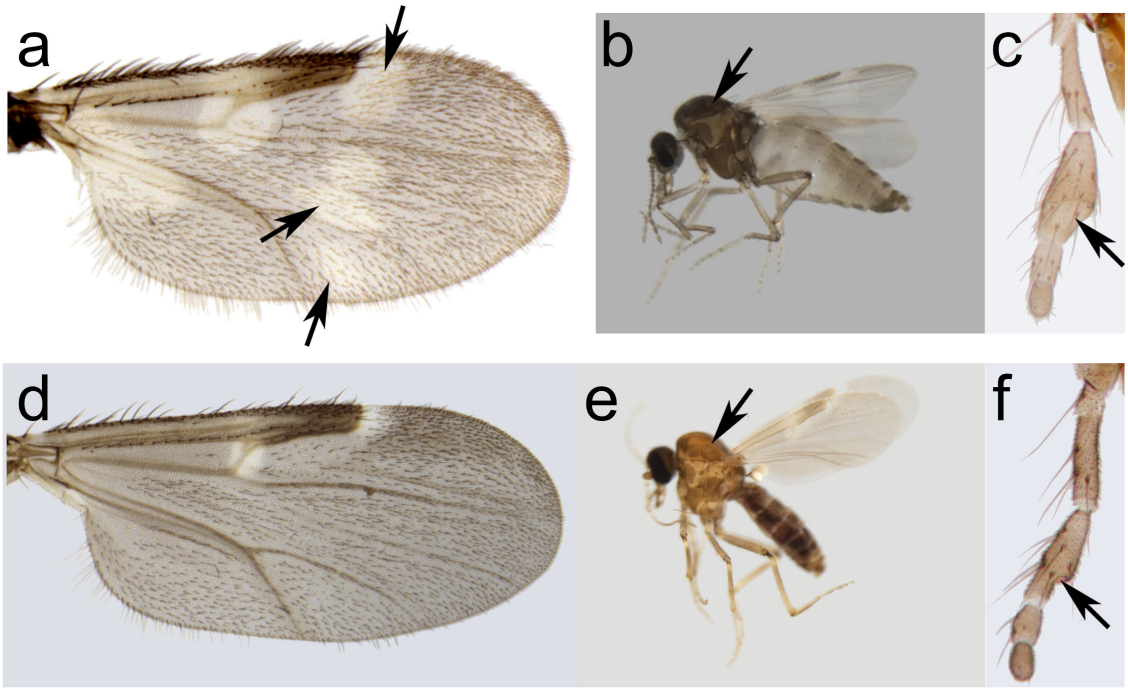

FIGURE 38 (a–f). Wing, body and palpus of *C. pallidicornis* (a–c), and *C. biguttatus* (d–f)

39 (38). Third palpal segment greatly swollen with small, deep sensory pit (Fig. 39-a,b); wing with several distinct pale spots in addition to pale spots over R-M crossvein and poststigmatic pale spot (Fig. 39-c). 40

Third palpal segment slightly to moderately swollen with shallower pit (Fig. 39-d,e); wing with or without additional pale spots (Fig. 39-f) 41

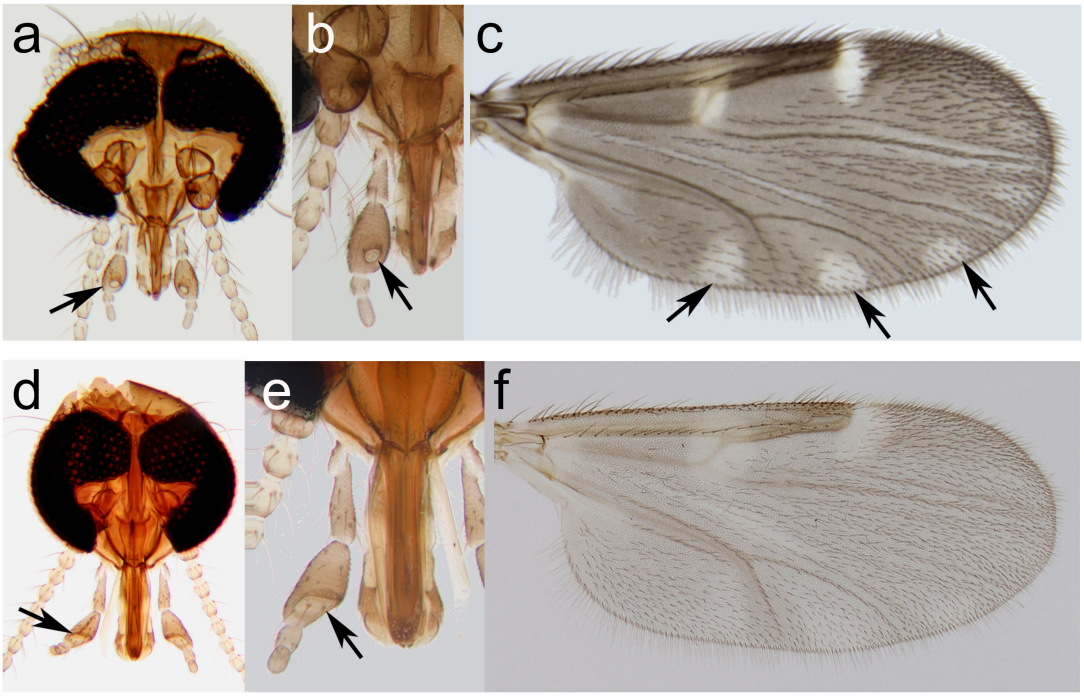

FIGURE 39 (a–f). Head, palpus and wing of *C. nanus* (a–c), and *C. travisi* (d–f)

40 (39). Wing with distinct pattern of small pale spots at the apices of cells m_1, m_2, cua_1, anal cell and often r_3 (Fig. 40-a); proboscis very short (P/H ratio 0.57) (Fig. 40-b); spermathecae lacking necks (Fig. 40-c). . . . . *C. nanus*

Wing lacking pale spots at the apices of cells r_3 and m_1 (Fig. 40-d); proboscis not so short (P/H ratio 0.68) (Fig. 40-e); spermathecae with slender necks (Fig. 40-f) . . . . . *C. footei*

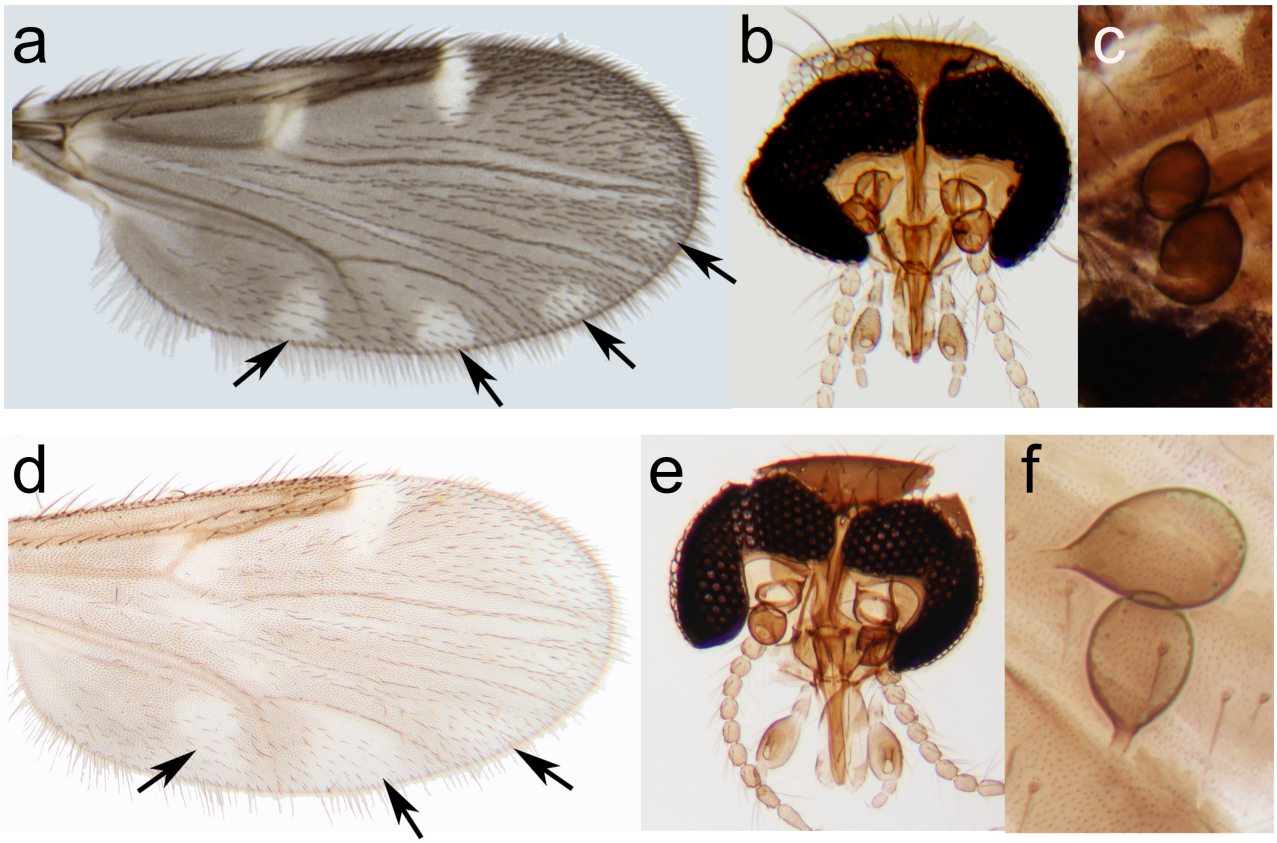

FIGURE 40 (a–f). Wing, head and spermathecae of *C. nanus* (a–c), and *C. footei* (d–f),

41 (39). Wing rather long and narrow, with pale spots appearing as light streaks between the darker veins; macrotrichia sparse in distal two-thirds of wing, on wing veins, in rows parallel to wing veins and scattered in cells (Fig. 41-a); one spermatheca; eyes broadly separated; sparsely distributed on Florida coasts . . . . . *C. bermudensis*

Wing not long and narrow, most pale spots rounded, not streak-like; macrotrichia variable (Fig. 41-b); two spermathecae; eye separation variable; not coastal 42

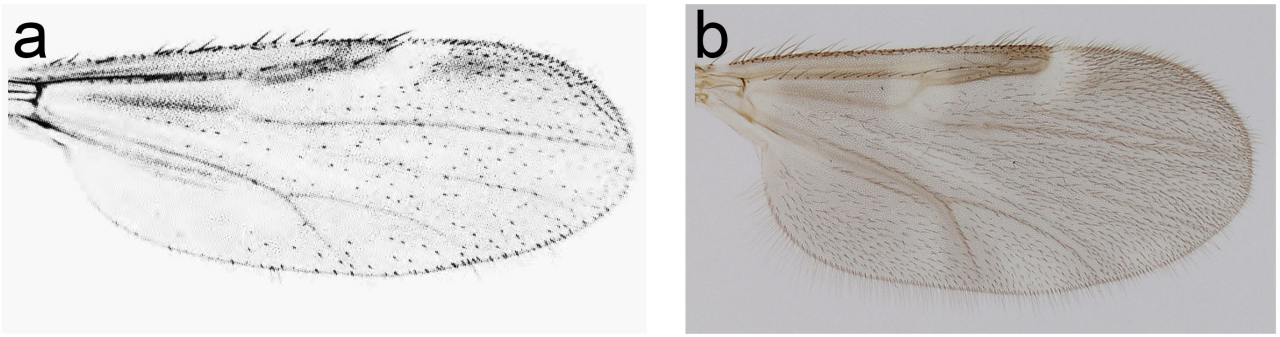

FIGURE 41 (a–b). Wing of *C. bermudensis* (a), and *C. travisi* (b)

42 (41). Vestigial mouthparts with very short proboscis (P/H ratio 0.49) and undeveloped tormae (Fig. 42-a); wing grayish brown, with indistinct pale spots and long, coarse macrotrichia (Fig. 42-b); uncommon in Florida. . . . . *C. loisae*

Mouthparts not vestigial, proboscis not so short (P/H ratio >0.65) and tormae present (Fig. 42-c); wing variable, but macrotrichia not long and coarse (Fig, 42-d). 43

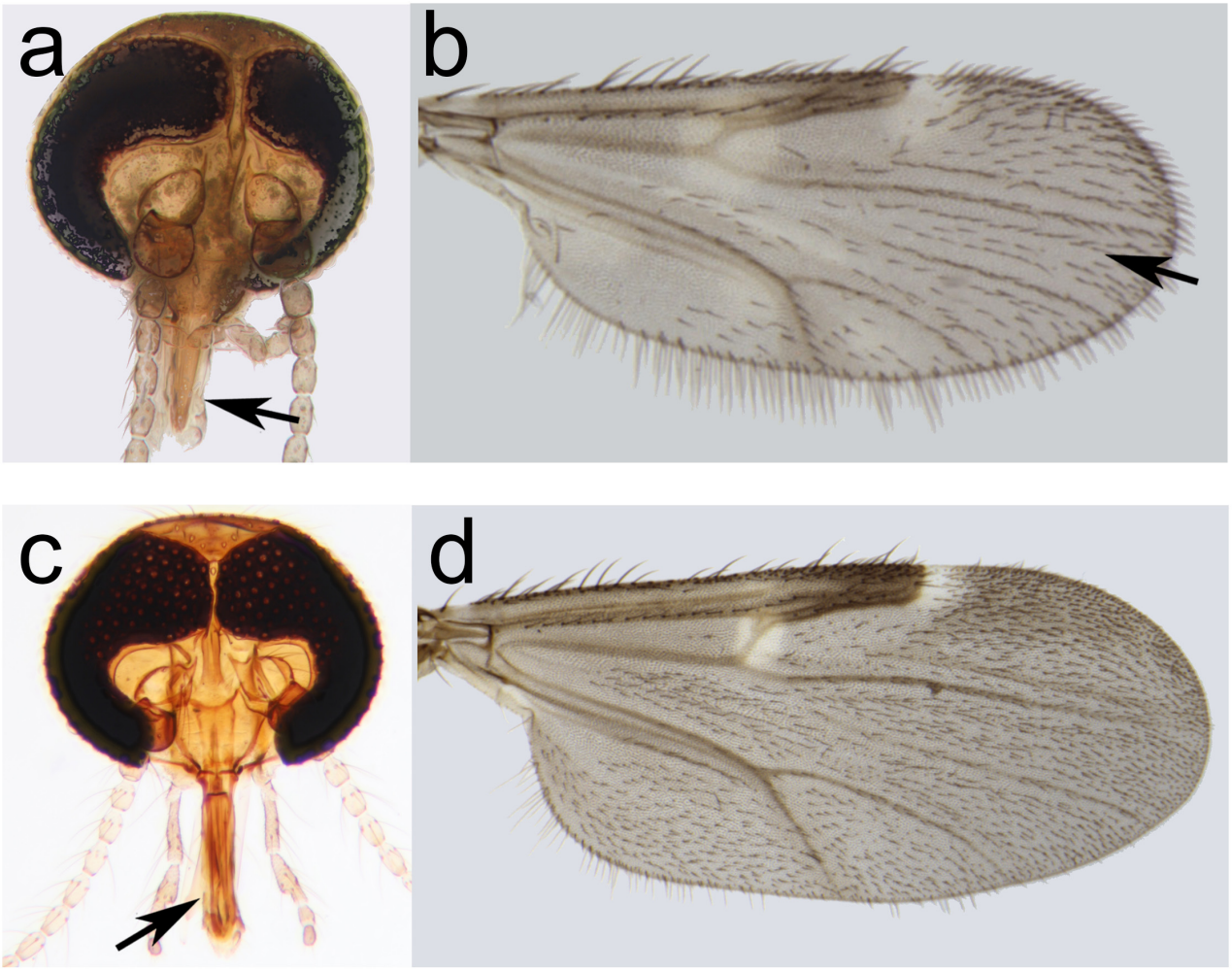

FIGURE 42 (a–d). Head and wing of *C. loisae* (a, b) and *C. biguttatus* (c, d)

43 (42). Wing with macrotrichia sparsely distributed on the distal half (Fig. 43-a). . . . . .*C. mulrennani*

Wing with microtrichia dense and widely distributed (Fig. 43-b) 44

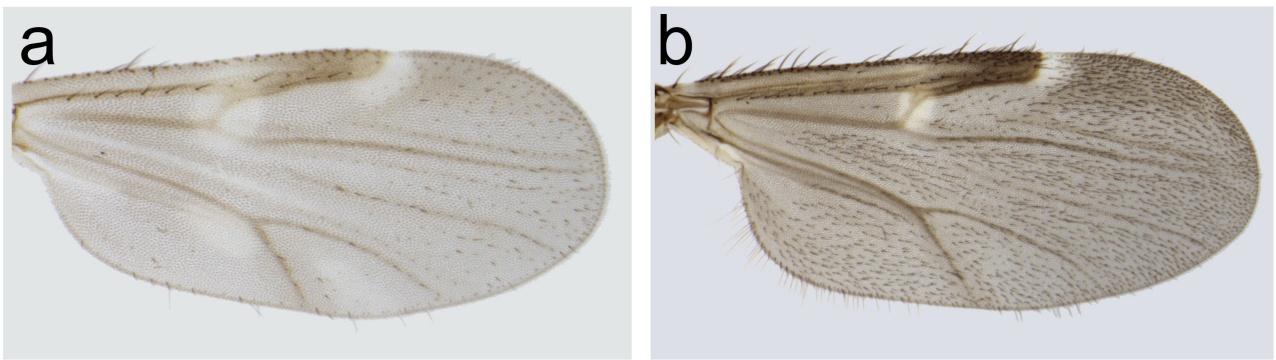

FIGURE 43 (a–b). Wing of *C. mulrennani* (a), and *C. biguttatus* (b)

44 (43). Wing with distinct pale spot or indistinct pale area at apices of two or more distal cells (r_3, m_1, or m_2) (Fig. 44-a,b). 45

Wing lacking pale spots or pale areas at the apices of cells r_3, m_1, and m_2 (Fig. 44-c,d) 46

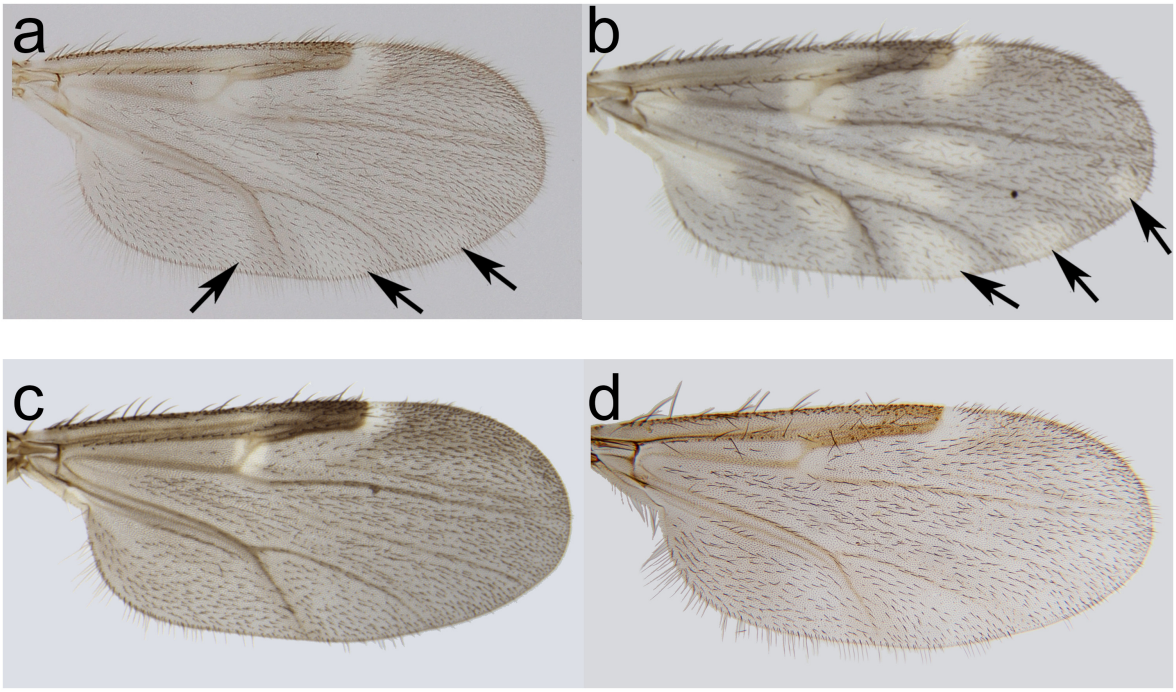

FIGURE 44 (a–d). Wing of *C. travisi* (a), *C. spinosus* (b), C. *biguttatus* (c) and *C. testudinalis* (d)

45 (44). Spermathecae ovoid with tapering necks (Fig. 45-a); sensory pit of third palpal segment irregular, shallow (Fig. 45-b); cell r_3 dark apically or with minute pale area at the extreme apex (Fig. 45-c). . . . . *C. spinosus*

Spermathecae oval and lacking tapering necks (Fig. 45-d); sensory pit of third palpal segment large, circular, shallow (Fig. 45-e); cell r_3 with elongate indistinct apical pale area (Fig. 45-f) . . . . . *C. travisi*

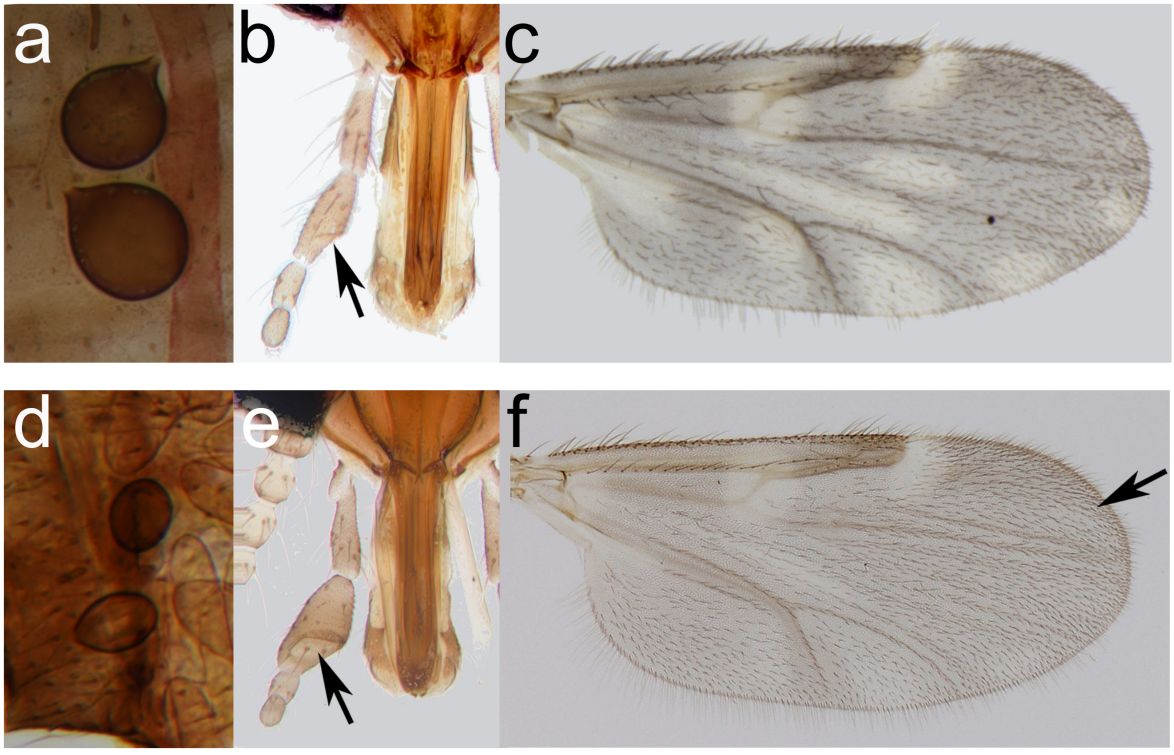

FIGURE 45 (a–f). Spermathecae, palpus and wing of *C. spinosus* (a–c), and *C. travisi* (d–f)

46 (44). Spermathecae with tapering necks and lacking sclerotized rings (Fig. 46-a); sensory pit of third palpal segment irregular (Fig. 46-b); wing with small, distinct pale spots over R-M crossvein and at apex of second radial cell, otherwise dark grayish brown (Fig. 46-c). . . . . *C. biguttatus*

Spermathecae with sclerotized rings but lacking tapering necks (Fig. 46-d); sensory pit of third palpal segment circular (Fig. 46-e); pale spots on wing mostly large and/or indistinct (Fig. 46-f). 47

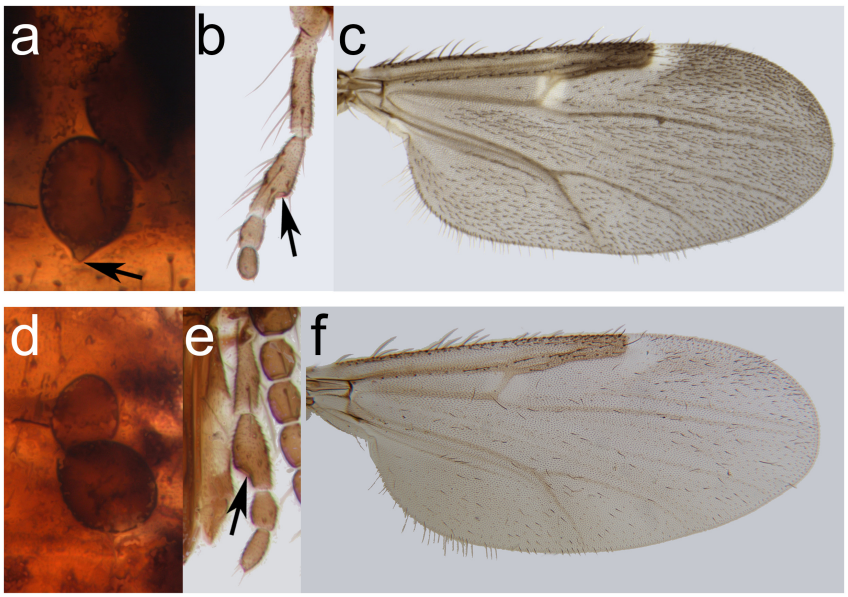

FIGURE 46 (a–f). Spermathecae, palpus and wing of *C. biguttatus* (a–c), and *C. husseyi* (d–f)

47 (46) Terminal two antennal segments broad, distinctly tapered apically (Fig. 47-a); pale spot over R-M cross vein distinct (Fig. 47-b). . . . . *C. testudinalis*

Terminal antennal segments narrow, not distinctly tapered apically (Fig. 47-c); pale spot over R-M cross vein indistinct (Fig. 47d). . . . . *C. husseyi*

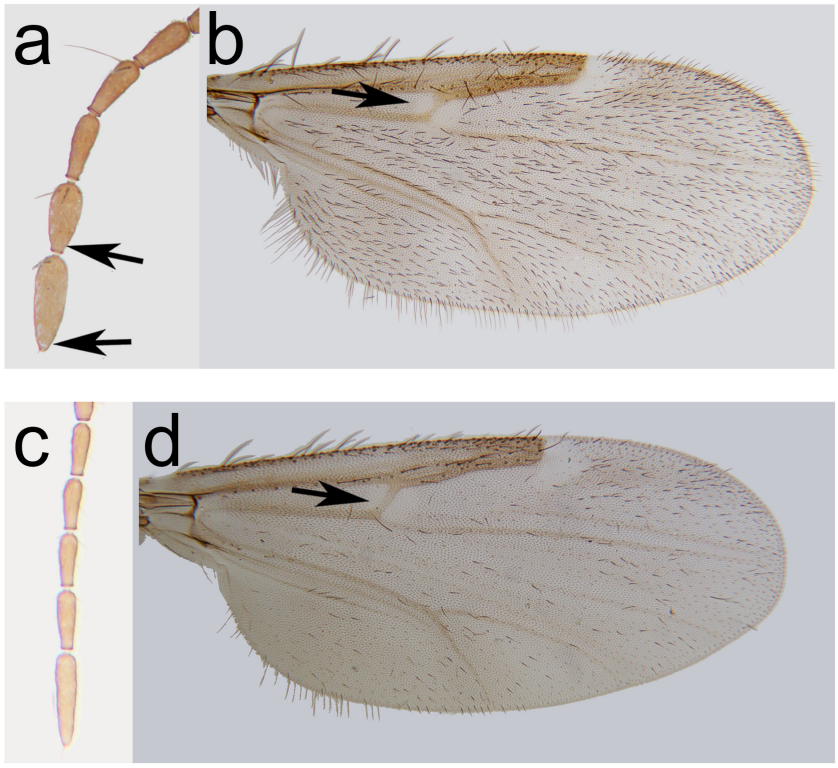

FIGURE 47 (a–d). Terminal antennal segments and wing of *C. testudinalis* (a, b), and *C. husseyi* (c, d)

TABLE 1. Distribution, abundance, and seasonality of *Culicoides* spp. in Florida, USA. Information compiled from Blanton and Wirth (1979), Kramer *et al*. (1985), Wilkening *et al*. (1985), Kline (1986), Vigil *et al*. (2014).

Subgenus	Species	Authority	Distribution	Abundance	Seasonality
Amossovia Glukhova	*arboricola*	Root and Hoffman	Statewide	Common	Year-round
	beckae	Wirth and Blanton	Northern Florida	Uncommon	Spring through fall
	guttipennis	(Coquillett)	Northern Florida	Uncommon	Spring through fall
	ousairani	Khalaf	Northern Florida	Rare	Spring through fall
	villosipennis	Root and Hoffman	North and central Florida	Uncommon	Spring through fall
Avaritia Fox	*alachua*	Jamnback and Wirth	Peninsular uplands	Uncommon	Spring
	chiopterus	(Meigen)	Scattered localities	Rare	Spring
	juddi	Cochrane	Northern Florida	Rare	Spring through fall
	pechumani	Cochrane	Northern Florida	Rare	Spring
	pusillus	Lutz	Peninsula	Rare	Winter and spring
Beltranmyia Vargas	*bermudensis*	Williams	Coastal peninsula	Uncommon	Spring and summer
	crepuscularis	Malloch	Statewide	Common	Spring through fall
	hollensis	(Melander and Brues)	Coastal	Common	Fall through spring
	knowltoni	Beck	Central Florida	Common	Year-round
	mississippiensis	Hoffman	Gulf Coast	Common	Year-round
Diphaomyia Vargas	*baueri*	Hoffman	North and central Florida	Uncommon	Spring
	edeni	Wirth and Blanton	Central and southern Florida	Common	Spring through fall
	footei	Wirth and Jones	Northern Florida	Rare	Spring
	haematopotus	Malloch	Northern Florida	Common	Spring through fall
Drymodesmyia Vargas	*hinmani*	Khalaf	Northern Florida	Common	Spring through fall
	jamaicensis	Edwards	Southern Florida coast	Uncommon	Summer
	loughnani	Edwards	Coastal peninsula	Uncommon	Spring and summer
Haematomyidium Goeldi	*debilipalpis*	Lutz	Scattered localities	Locally common	Summer
	paraensis	(Goeldi)	North and central Florida	Locally common	Spring through fall

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TABLE 1. (continued)

Subgenus	Species	Authority	Distribution	Abundance	Seasonality
	torreyae	Wirth and Blanton	North and central Florida	Rare	Spring and fall
Hoffmania Fox	*insignis*	Lutz	Statewide	Common	Summer and fall
	venustus	Hoffman	Northern Florida	Common	Spring through fall
Monoculicoides Khalaf	*sonorensis*	Wirth and Jones	Northern Florida	Rare	Spring through fall
	variipennis	(Coquillett)	Northern Florida	Rare	Spring through fall
Oecacta Poey	*barbosai*	Wirth and Blanton	Southern Florida coast	Common	Spring through fall
	furens	(Poey)	Coastal	Common	Year-round
	stellifer	(Coquillett)	North and central Florida	Common	Spring through fall
Silvaticulicoides Glukhova	*biguttatus*	(Coquillett)	Northern Florida	Common	Spring
	loisae	Jamnback	Northern Florida	Uncommon	Spring and summer
	mulrennani	Beck	Northern Florida	Uncommon	Spring
	spinosus	Root and Hoffman	North and central Florida	Common	Spring and fall
Unplaced, Piliferus Species Group	*bickleyi*	Wirth and Hubert	Scattered localities	Rare	Spring
	husseyi	Wirth and Blanton	Northern Florida	Rare	Spring
	parapiliferus	Wirth and Blanton	Northern Florida	Rare	Spring
	piliferus	Root and Hoffman	Northern Florida	Rare	Spring
	scanloni	Wirth and Hubert	North and central Florida	Common	Spring
	snowi	Wirth and Jones	Western Florida	Uncommon	Spring and fall
	testudinalis	Wirth and Hubert	Northern Florida	Rare	Spring
Unplaced, Stonei Species Group	*melleus*	(Coquillett)	Coastal	Common	Spring and summer
	pallidicornis	Kieffer	North and central Florida	Common	Winter and spring
	tissoti	Wirth and Blanton	Northern Florida	Locally common	Spring
Miscellaneous Unplaced Species	*floridensis*	Beck	Peninsula	Common	Summer
	nanus	Root and Hoffman	North and central Florida	Uncommon	Spring and summer
	travisi	Vargas	Scattered localities	Uncommon	Spring

TABLE 2. Summary of vectors and biting nuisance *Culicoides* spp. in Florida, USA. Information compiled from various sources.

Pathogen / importance	*Culicoides* species
Avian Haemosporida (*Plasmodium*, *Haemoproteus*)	*C. arboricola*, *C. crepuscularis*, *C. edeni*, *C. haematopotus*, *C. hinmani*, *C. knowltoni*
Epizootic hemorrhagic disease virus	*C. debilipalpis*, *C. insignis*, *C. sonorensis*, *C. stellifer*, *C. variipennis*, *C. venustus*
Bluetongue virus	*C. insignis*, *C. sonorensis*
Biting nuisance	*C. barbosai*, *C. furens*, *C. hollensis*, *C. mississippiensis*, *C. tissoti*

Discussion

While relatively unambiguous characters are available for many of the first 38 couplets, correct identification of *Culicoides* spp. becomes increasingly challenging toward the end of this key (couplets 37-47), using stereoscopic examination. Species in the subgenus *Silvaticulicoides* that occur in Florida (*Culicoides biguttatus* (Coquillett), *Culicoides loisae* Jamnback, *Culicoides mulrennani* Beck, *Culicoides spinosus* Root and Hoffman) and two members of the piliferus species group (*Culicoides husseyi* Wirth and Blanton and *Culicoides testudinalis* Wirth and Hubert) have reduced or faint markings on the wings that are difficult to discern. In Florida, these species are mainly active in spring (April and May), are more common in northern than southern portions of the state, and are not considered important nuisance or vector species (Tables 1 and 2). Reliable identification of these species may still require used of compound microscopy to examine characters, particularly the spermatheca and maxillary palp (couplets 40-47). Other challenging portions of the key include separation of the four members of the piliferus group in Florida (couplets 33-35) that have distinct patterns of pale spots on the wings (*Culicoides bickleyi* Wirth and Hubert, *Culicoides parapiliferus* Wirth and Blanton, *Culicoides piliferus* Root and Hoffman, and *Culicoides scanloni* Wirth and Hubert). In general, the length of the proboscis relative to the median length of the head (P/H ration), the length of the distal antennal segments relative to the length of the proximal antennal segments (antennal ratio) and the shape of the third palpal segment are used to identify these species, typically using compound microscopy. Blanton and Wirth (1979) state that *C. scanloni* is by far the most common of these four species in Florida. *Culicoides bickleyi*, *C. parapiliferus*, and *C. piliferus* are northern (temperate) species and very few confirmed records exist for these species in Florida (Wilkening *et al*., 1985).

Culicoides mississippiensis and *C. hollensis* are closely related coastal species that were historically separated based upon distribution and degree of paleness in the wing. Blanton and Wirth (1979) state that *C. mississippiensis* occurs on the Gulf Coast and has 2nd radial cell pale in the distal portion, while *C. hollensis* occurs on the Atlantic Coast and has 2nd radial dark to its tip. Vigil *et al*. (2014), however reported that some *C. hollensis* specimens possessed a slight pale area over the 2nd radial cell and reported *C. hollensis* in southwest Florida (on the Gulf Coast) indicating that pigmentation of the radial cell and geographic distribution are unlikely to be definitive for separating these species. Blanton and Wirth (1979) suggested that should evidence of interbreeding be provided by future studies, that *C. mississippiensis* could be reduced to a subspecies of *C. hollensis*, as "structurally, there is little to separate it from *C. hollensis*."

Where appropriate, we have noted, in the couplets, species which are considered rare, restricted geographically, or restricted to certain habitats (coastal species, e.g.). This information is intended to help guide users of the key in gaining familiarity with expected patterns of occurrence but should not be relied upon as an identifying characteristic. New distribution records of seemingly rare species are needed, and these new records should be confirmed with microscopic characters that are explicitly described in Blanton and Wirth (1979).

The key provided here is undoubtedly imperfect yet provides numerous original images and additional resources to support future research on the ecology and vector potential of *Culicoides* spp. in Florida. Several nuisance and vector species (Table 2), such as *Culicoides insignis* Lutz, *Culicoides venustus* Hoffman, *Culicoides furens* (Poey), and *Culicoides stellifer* (Coquillett), have been brought "forward" in the key (couplets 5-9) which should facilitate correct identification of these species based upon characters of the wing. We recommend strongly that users of this

key also become familiarized with the keys and copious useful biological and morphological information provided in the comprehensive work by Blanton and Wirth (1979).

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